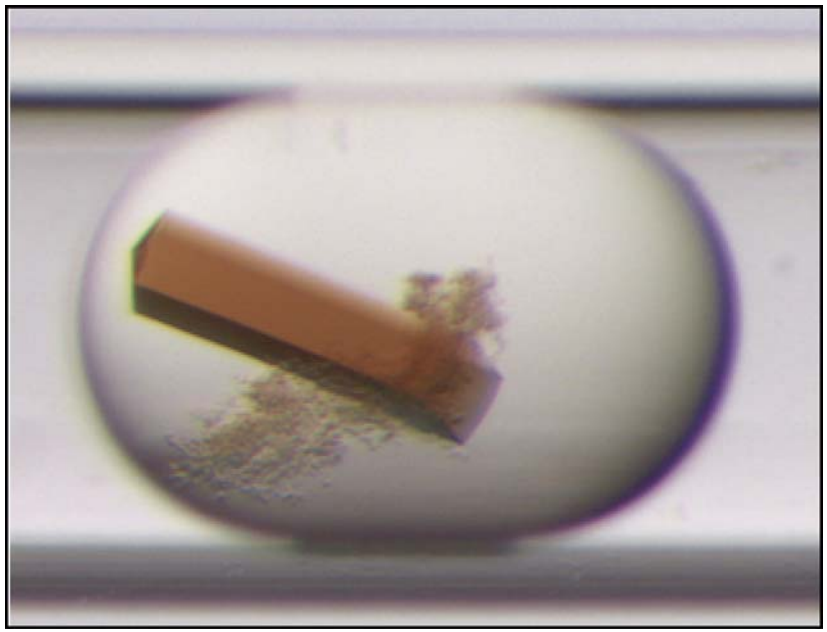
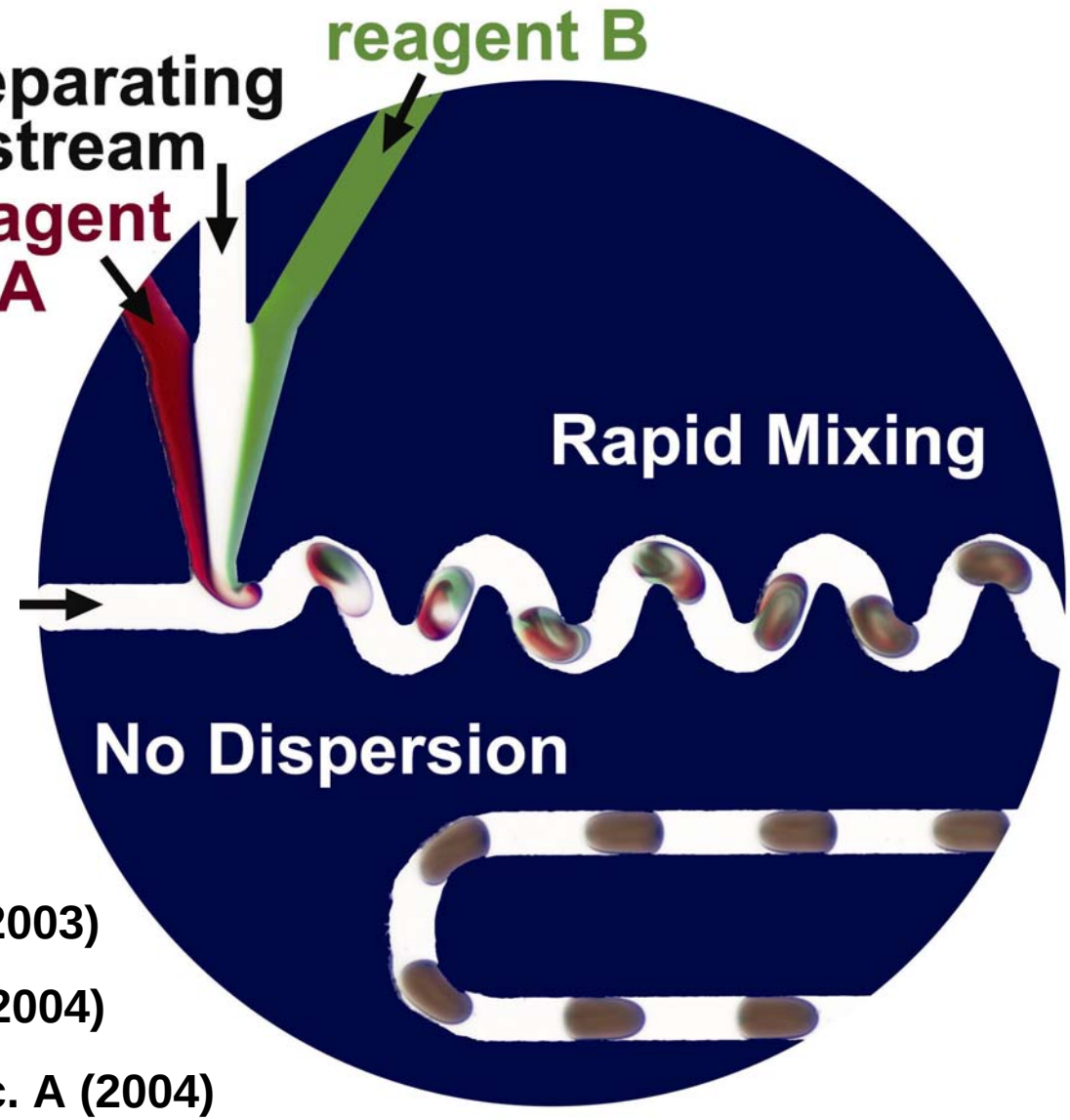
Crystallization of Membrane Proteins in Nanoliter Volumes Using Plug-based Microfluidics



Liang Li, Cory Gerdts, Debarshi Mustafi, Qiang Fu, Delai Chen
Rustem Ismagilov *
The University of Chicago
June 20th, 2007

Formation of Droplets (Plugs) in Microfluidics



Langmuir (2003)

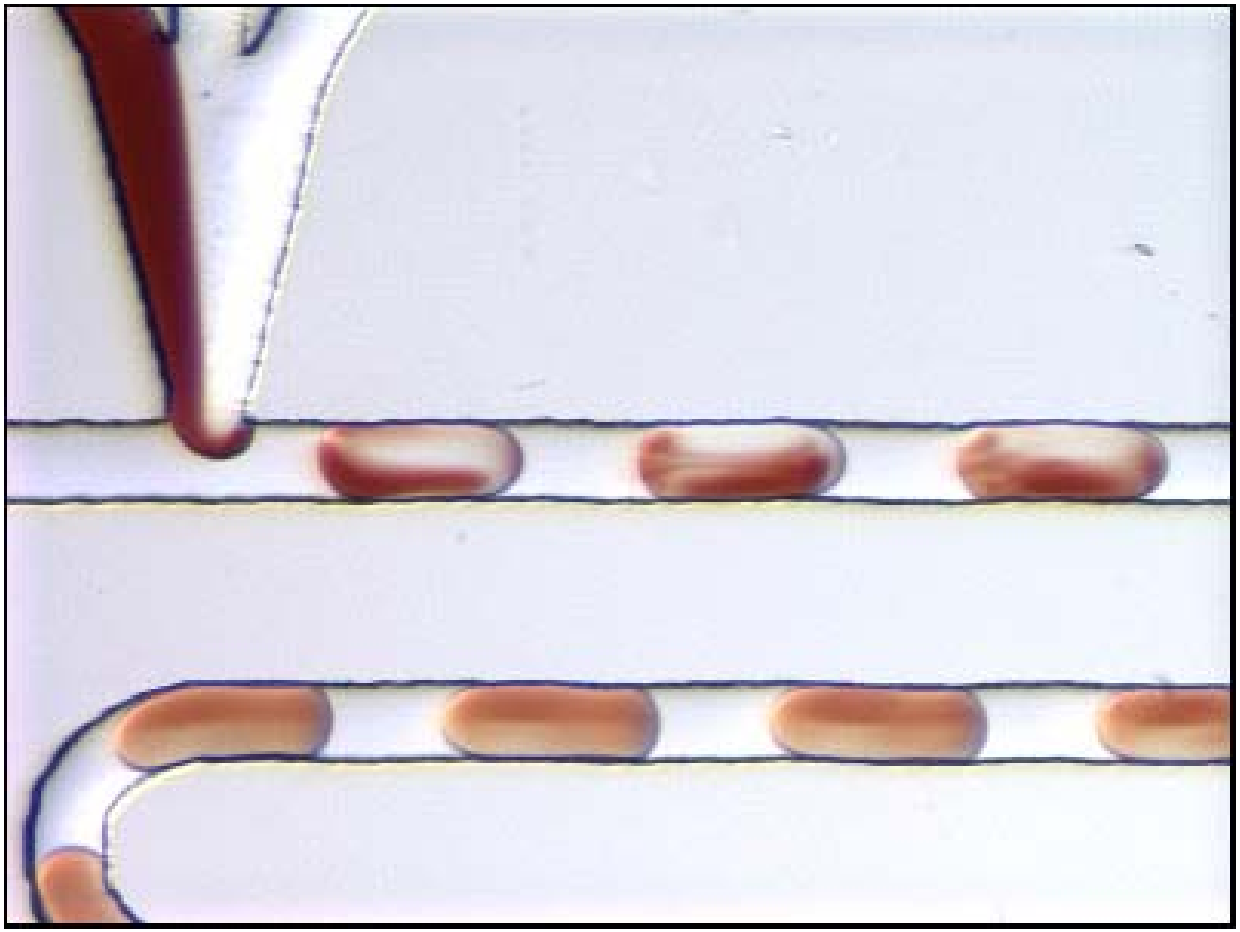
Appl. Phys. Lett. (2003)

Anal. Chim. Acta (2004)

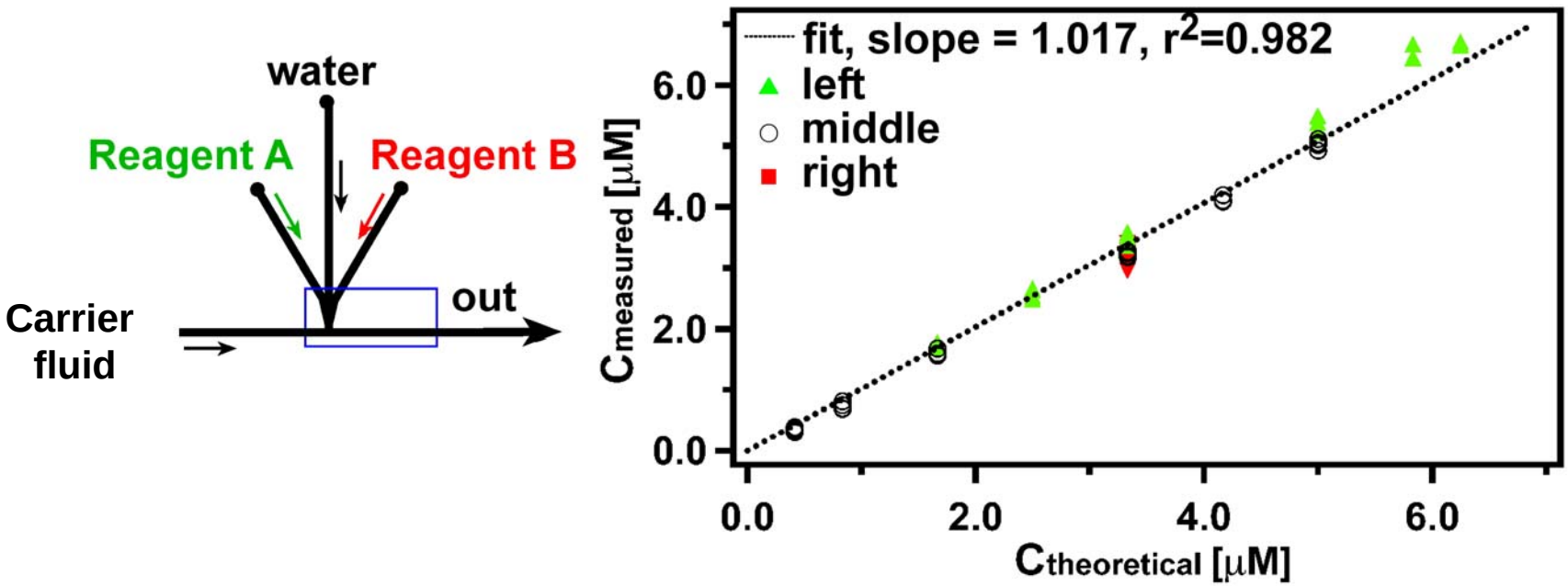
Philos. T. Roy. Soc. A (2004)

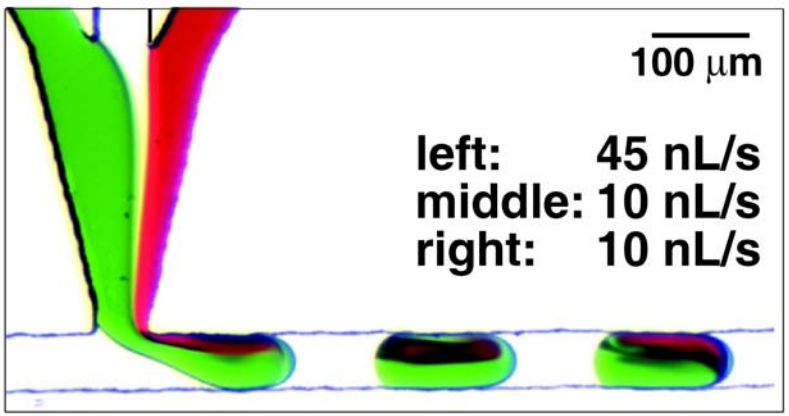
H. Song, J. D. Tice, R. F. Ismagilov *Angew. Chem.-Int. Edit.* 2003, 42, 768.

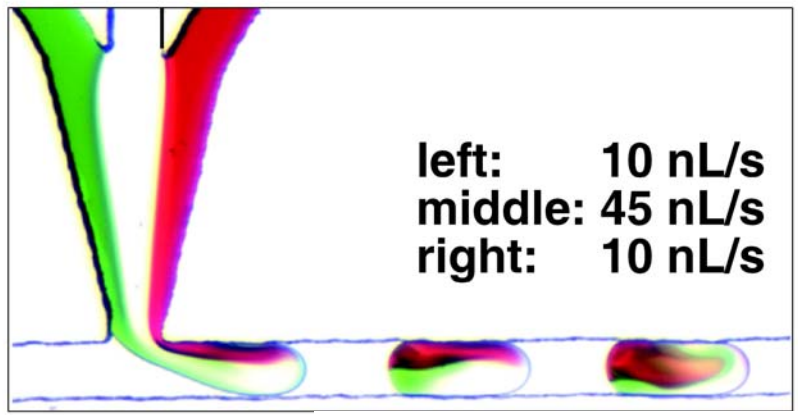
Eliminate Dispersion: Plugs Form in Fluorinated Carrier Fluid



Control Concentrations: Vary the Flow Rates

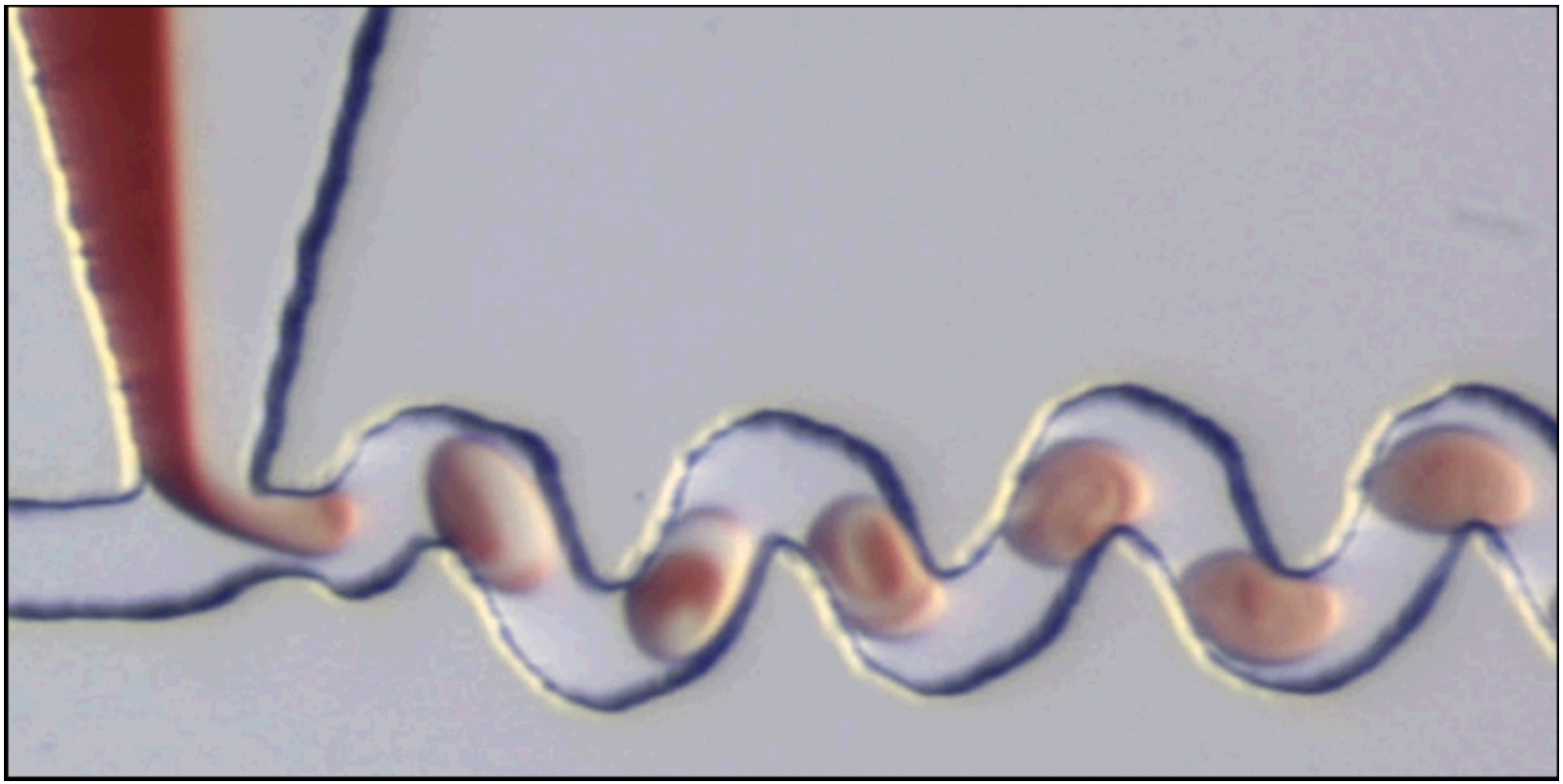




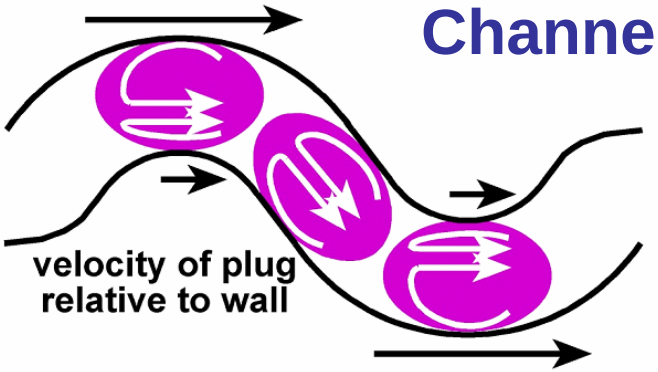


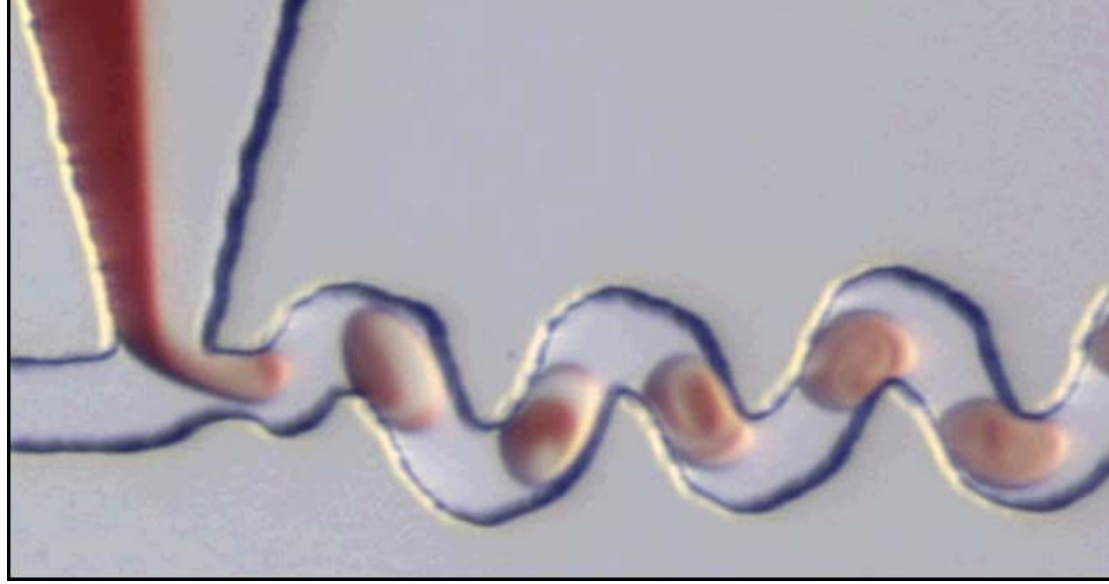
H. Song and R. F. Ismagilov *J. Am. Chem. Soc.* 2003, *125,* 14613.

Enhance Mixing Rate: Use Winding Channels

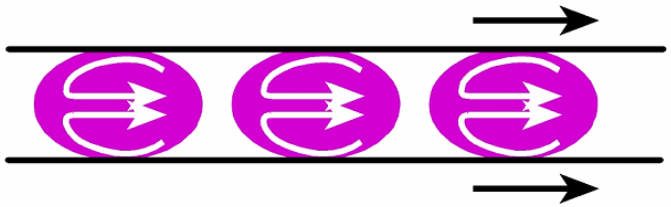


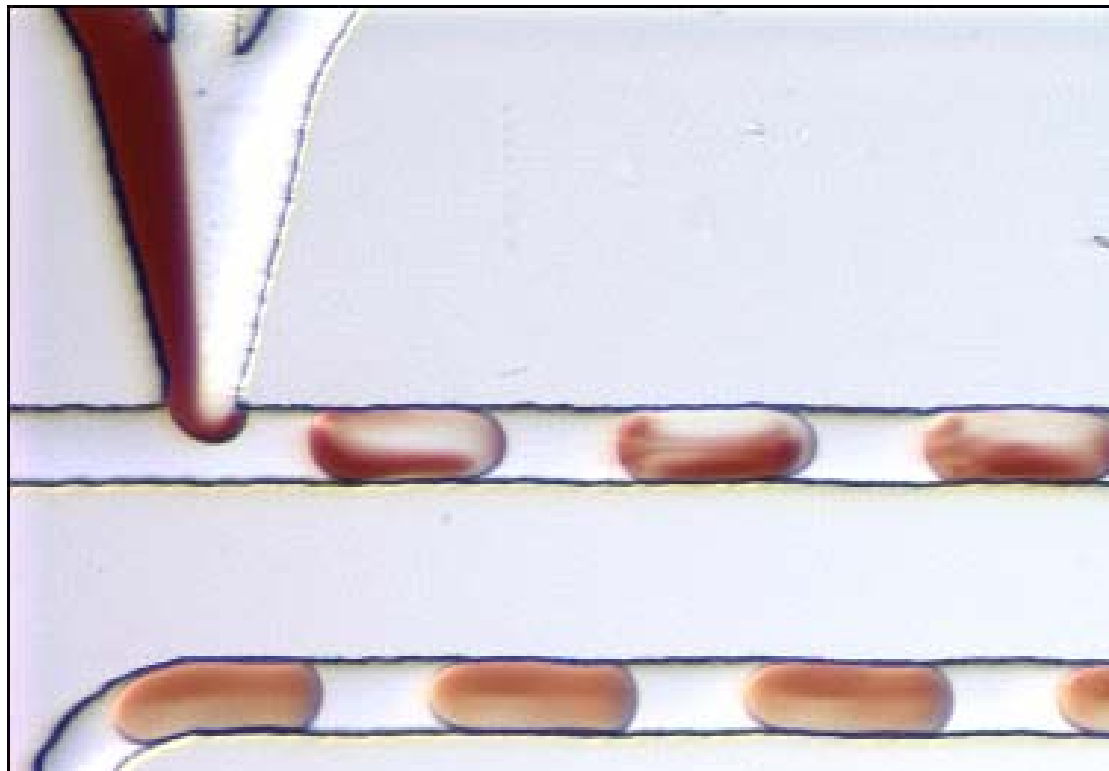
Rapid Mixing in Winding Channels





Slow Mixing in Straight Channels

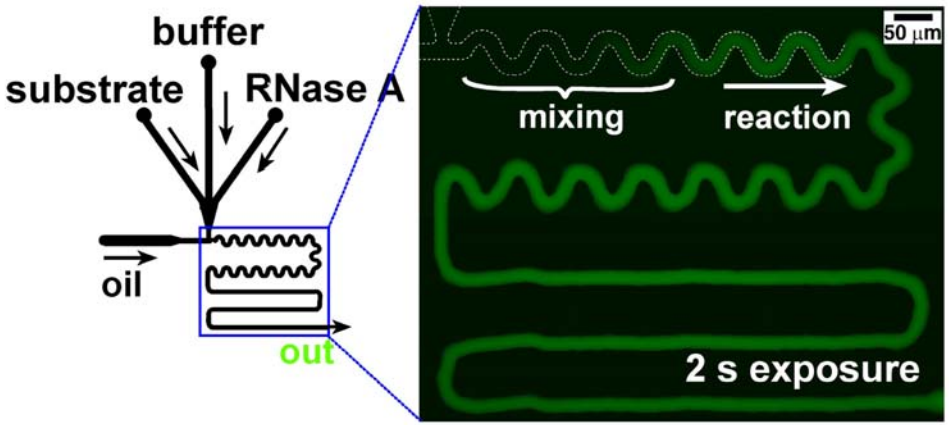




velocity of plug relative to wall

flow within plugs

Millisecond Kinetics Using Nanoliters of Solution

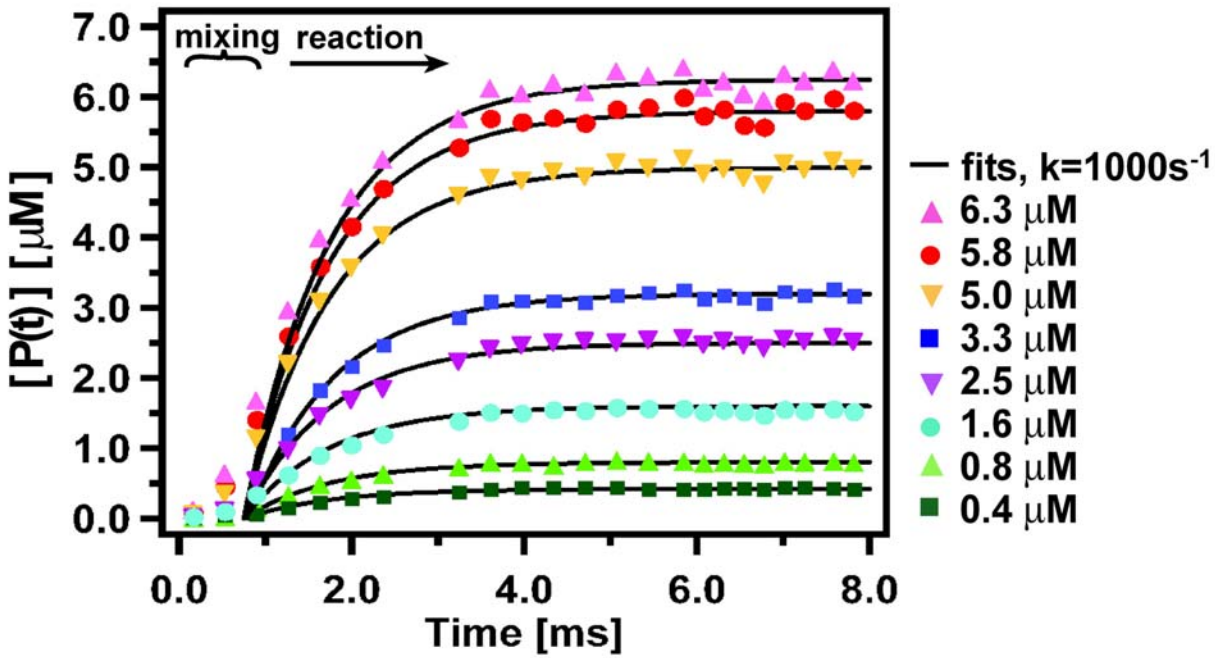


For single-turnover kinetics, where $[E]_0 >> [S]_0$:

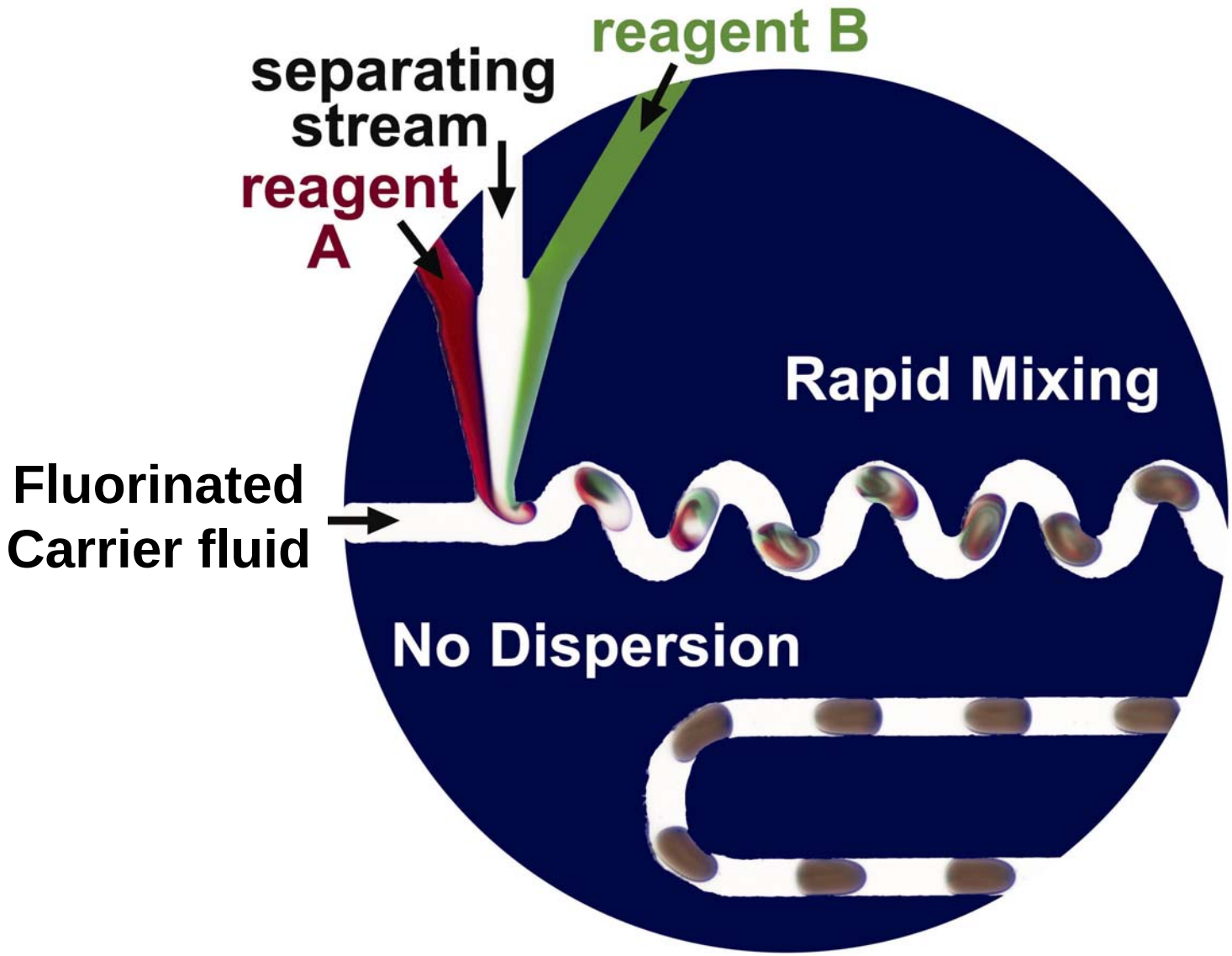
$$[P(t)] = [S]_0 (1 - Exp(-k \times t))$$

Sample consumption 33 nL/s

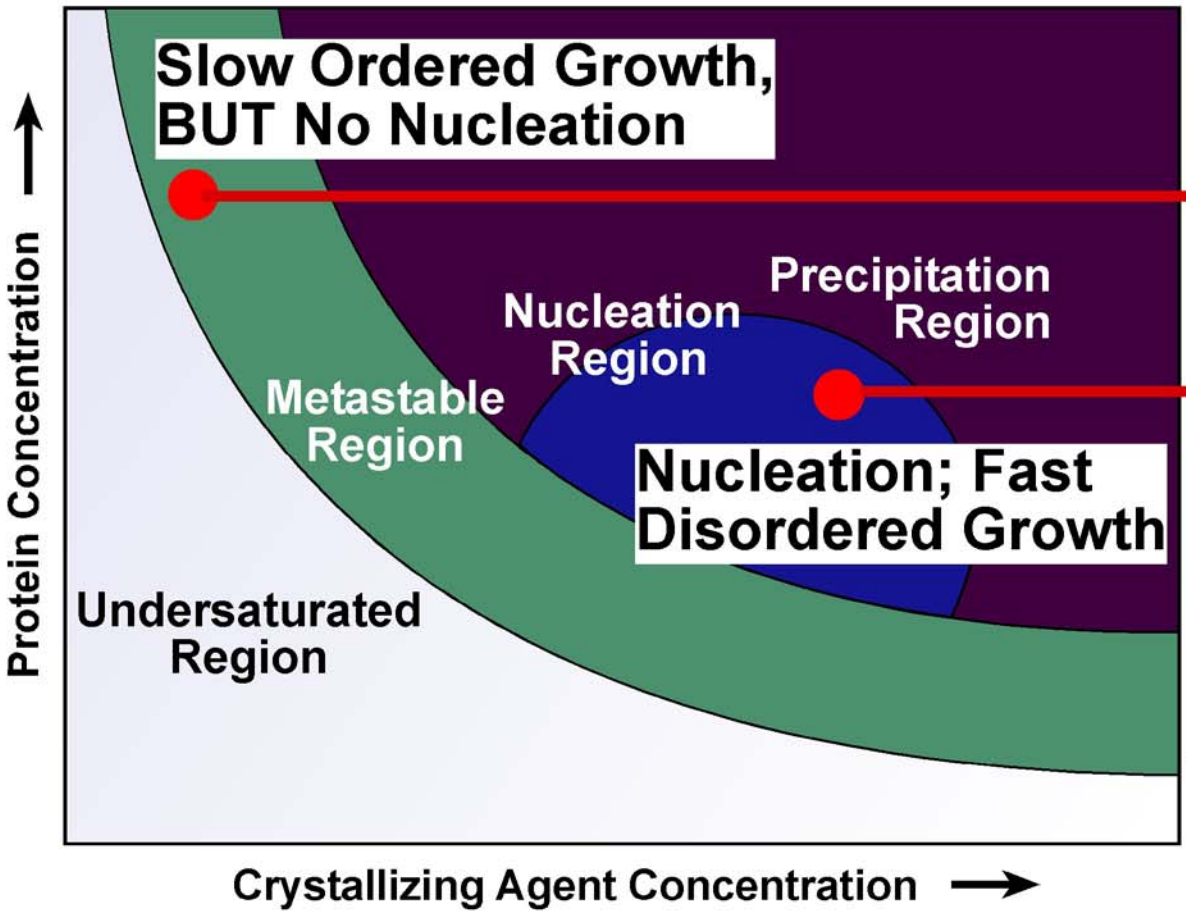
Used less than 1 μL total of sample for all 8 curves

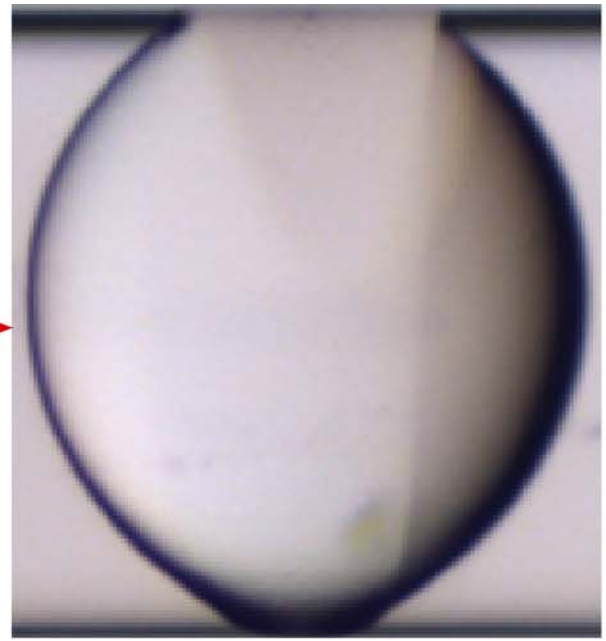


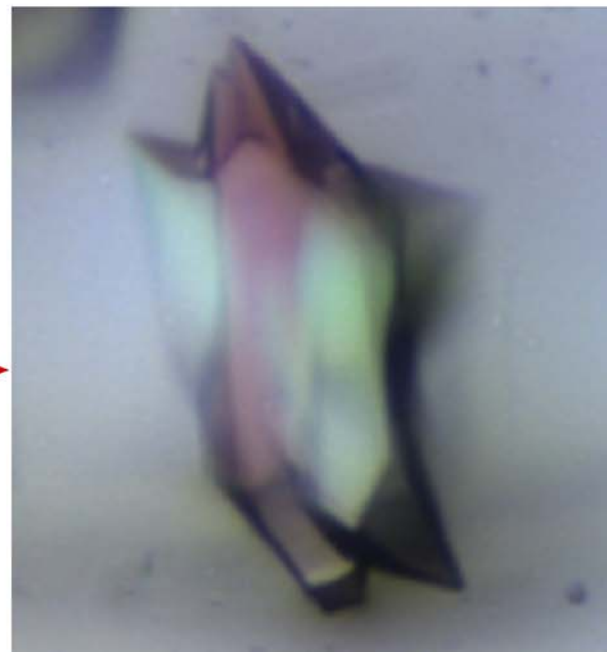
H. Song and R. F. Ismagilov *J. Am. Chem. Soc.* 2003, *125,* 14613-14619.


reagent B
separating stream
reagent A
Rapid Mixing
Fluorinated Carrier fluid
No Dispersion

Protein crystallization is to recognize the right crystallizing agent and to the right concentration

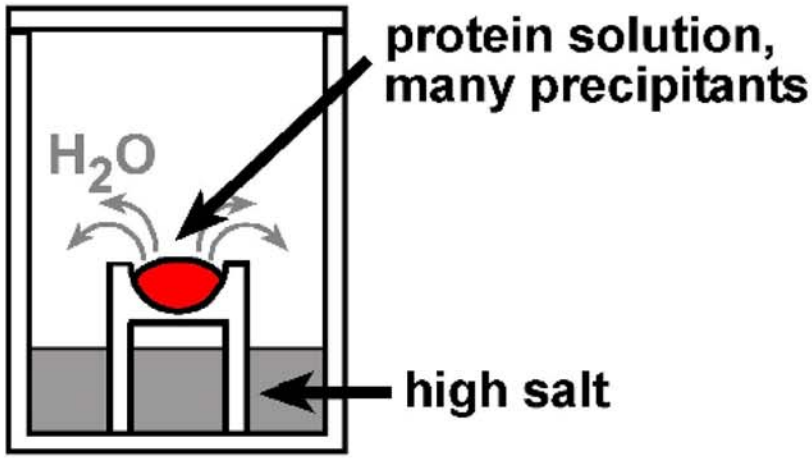




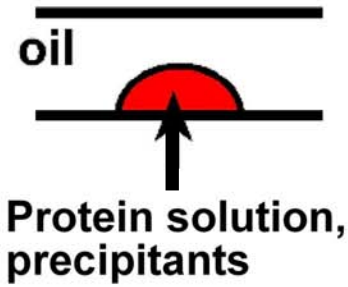


Protein Crystallization is Commonly Done in 100 -1000 nL (1 μL) droplets

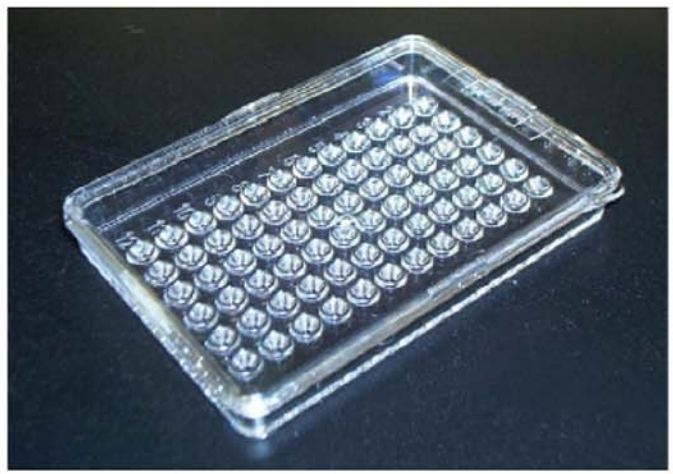
Vapor Diffusion



Microbatch



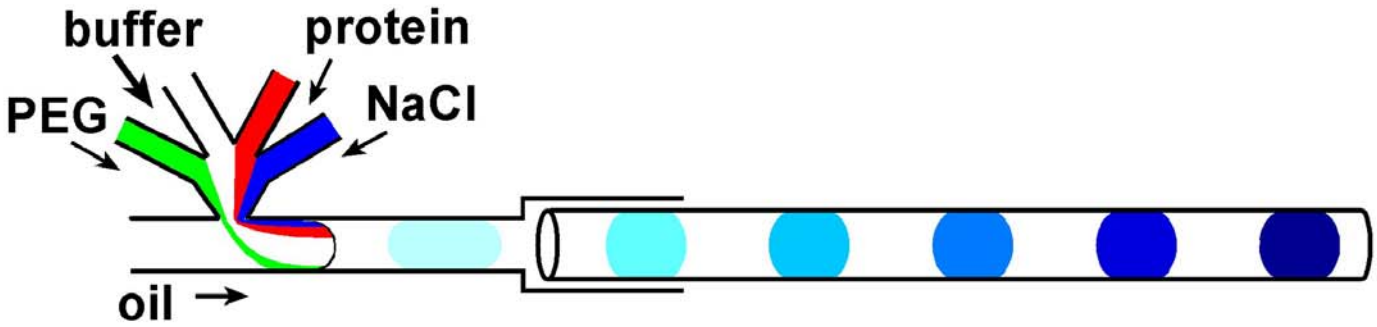
Microbatch Plate

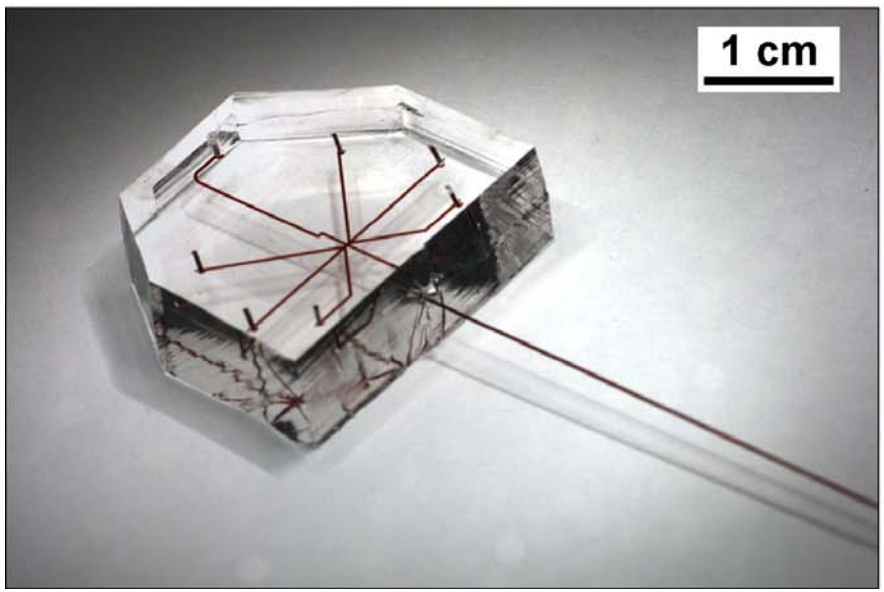


Optimal system:

-- Many trials
-- Low sample consumption
-- Rapid
-- Broad and dense coverage of space
-- No evaporation
-- Reproducibly generates trials
-- Reliably handles membrane protein

Crystallization in X-ray Capillaries: No Evaporation

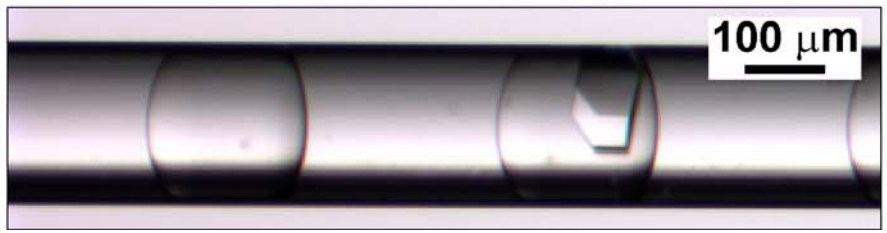




Form Plugs

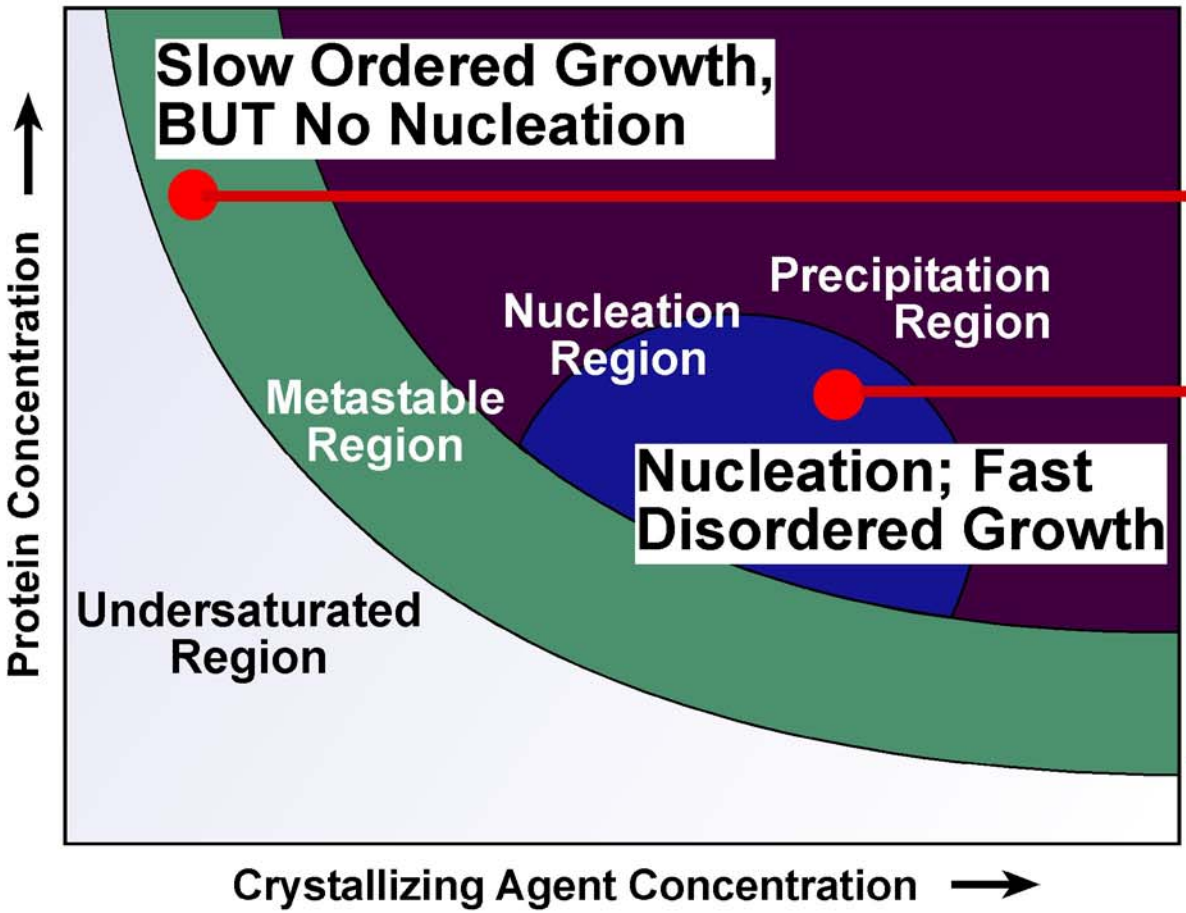
Detach / Seal Capillary

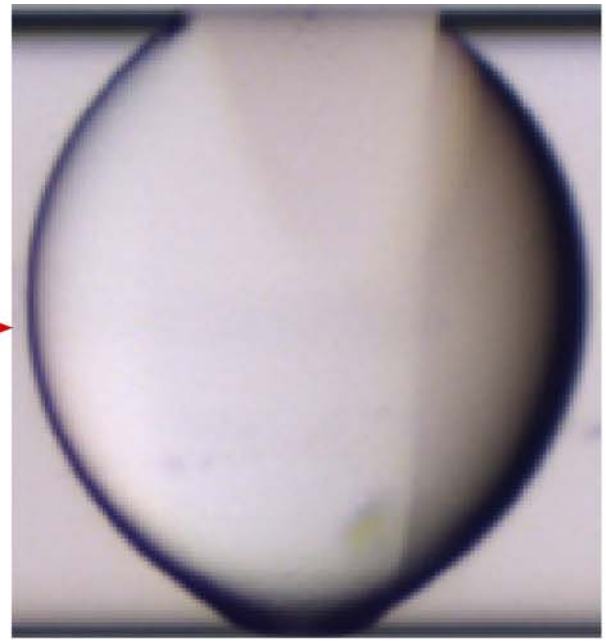
Incubate Capillary

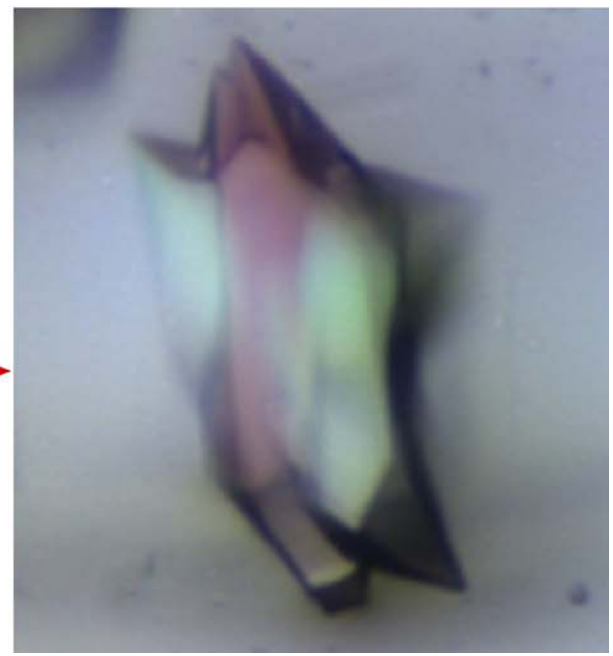


Stable for over a year

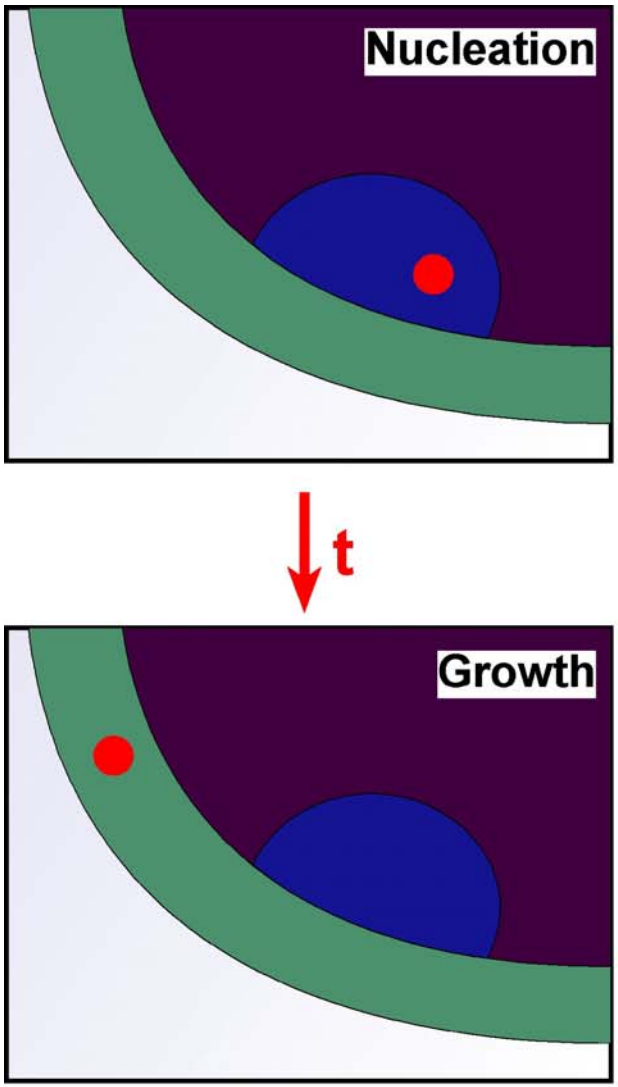
In crystallization
You get nucleation
Or ordered growth --
But rarely both

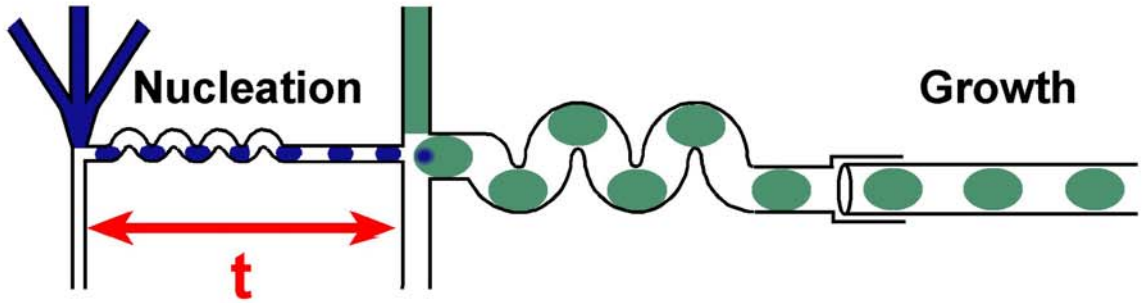


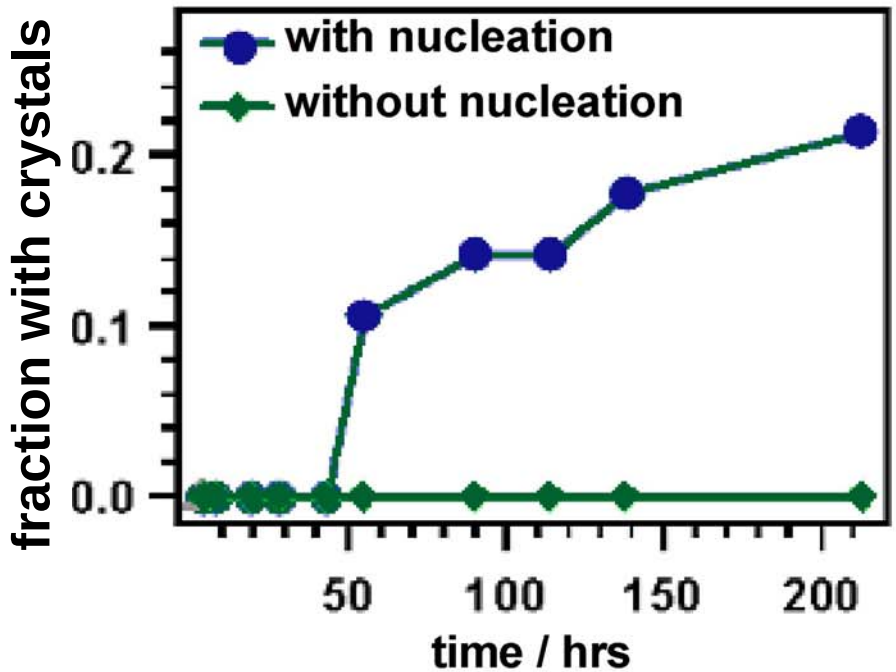




Independent Control of Nucleation and Growth Using Time and Concentration Control

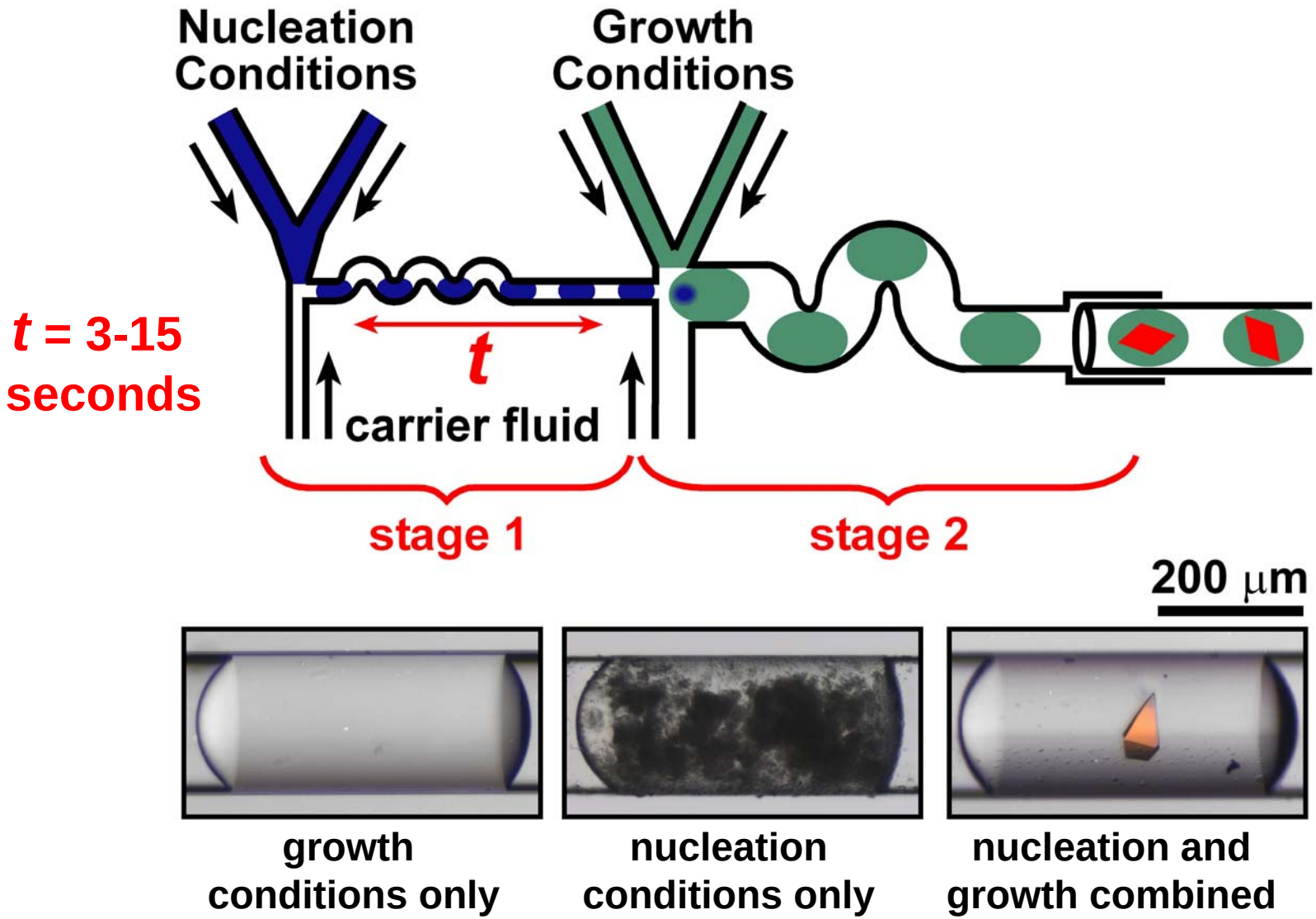




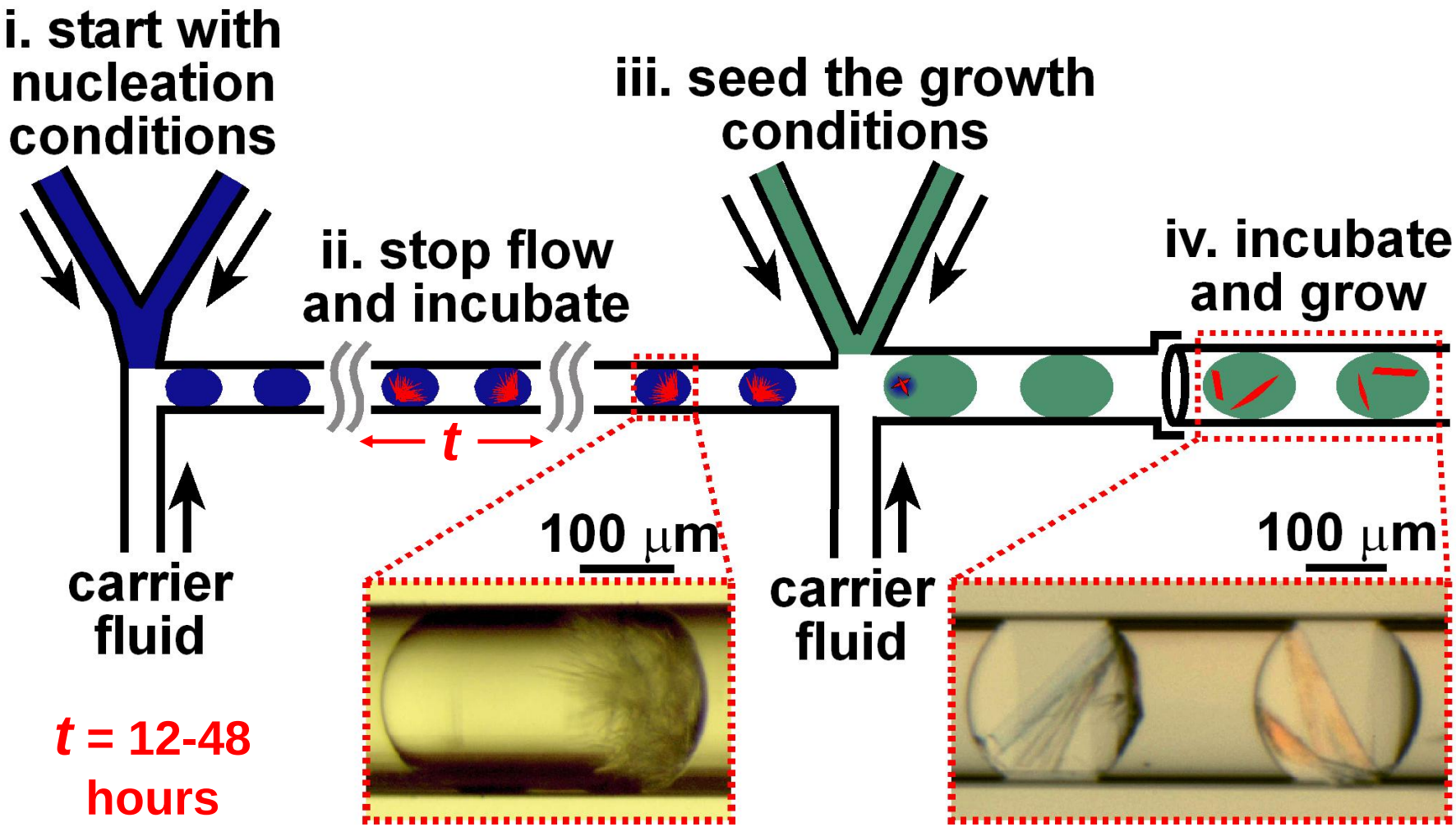


C. J. Gerdts and R.F.Ismagilov *Angew. Chem. Int. Ed.* 2006, 45,8156

Separation of Nucleation and Growth - Microfluidic Seeding

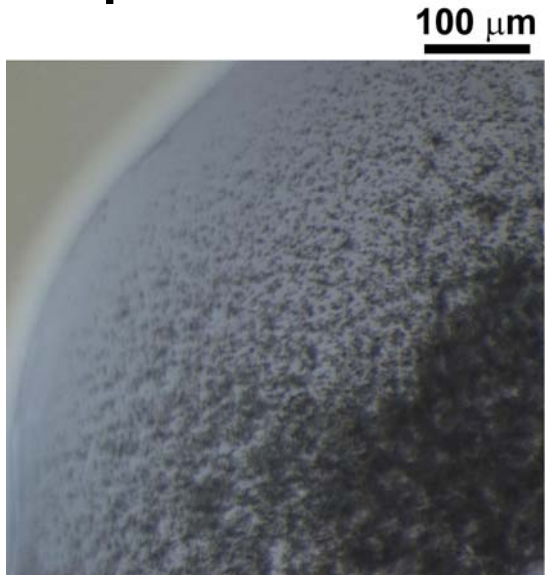


Microfluidic Seeding with SARS Nucleocapsid N-terminal Domain

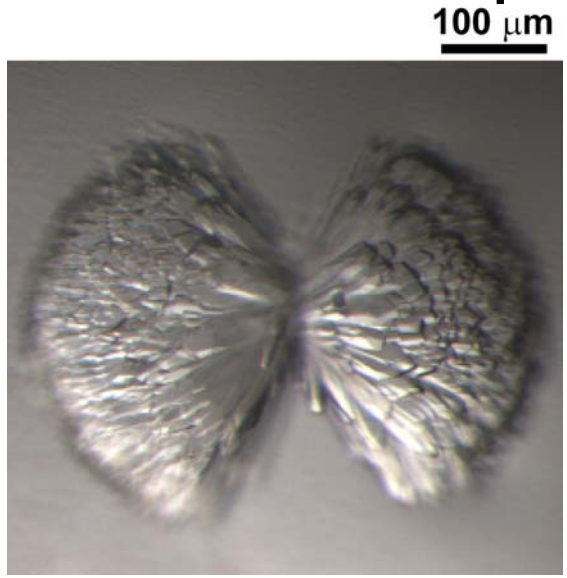


Crystallization of Oligoendopeptidase F - Microfluidic Seeding

Precipitation in VD drop

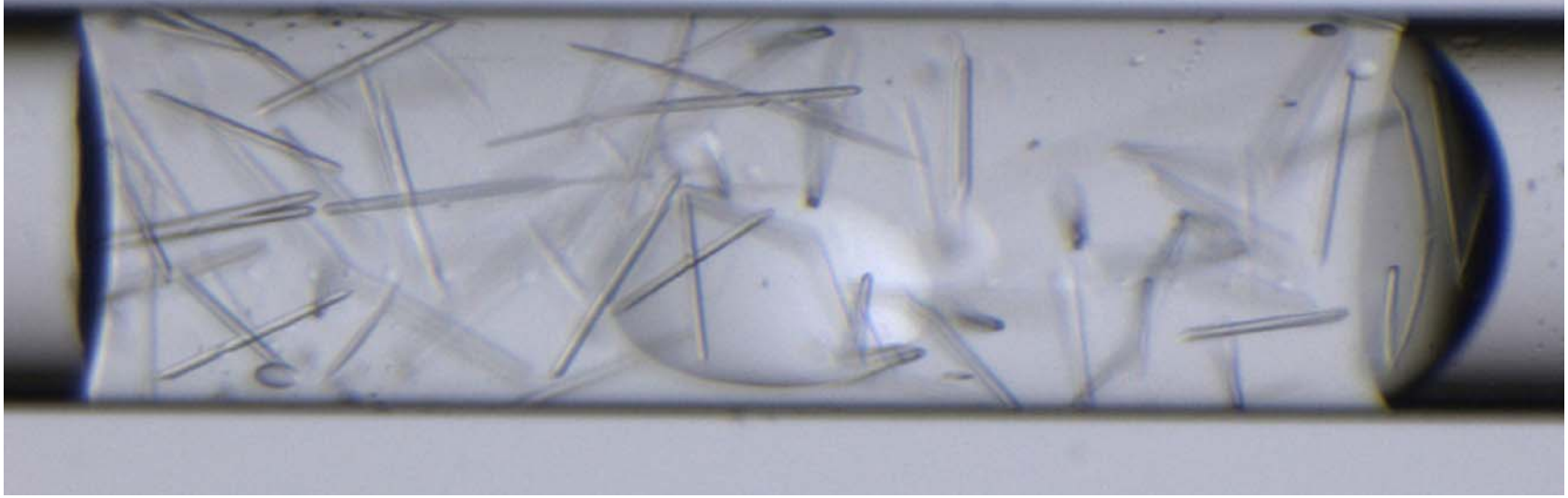


Clusters in VD drop



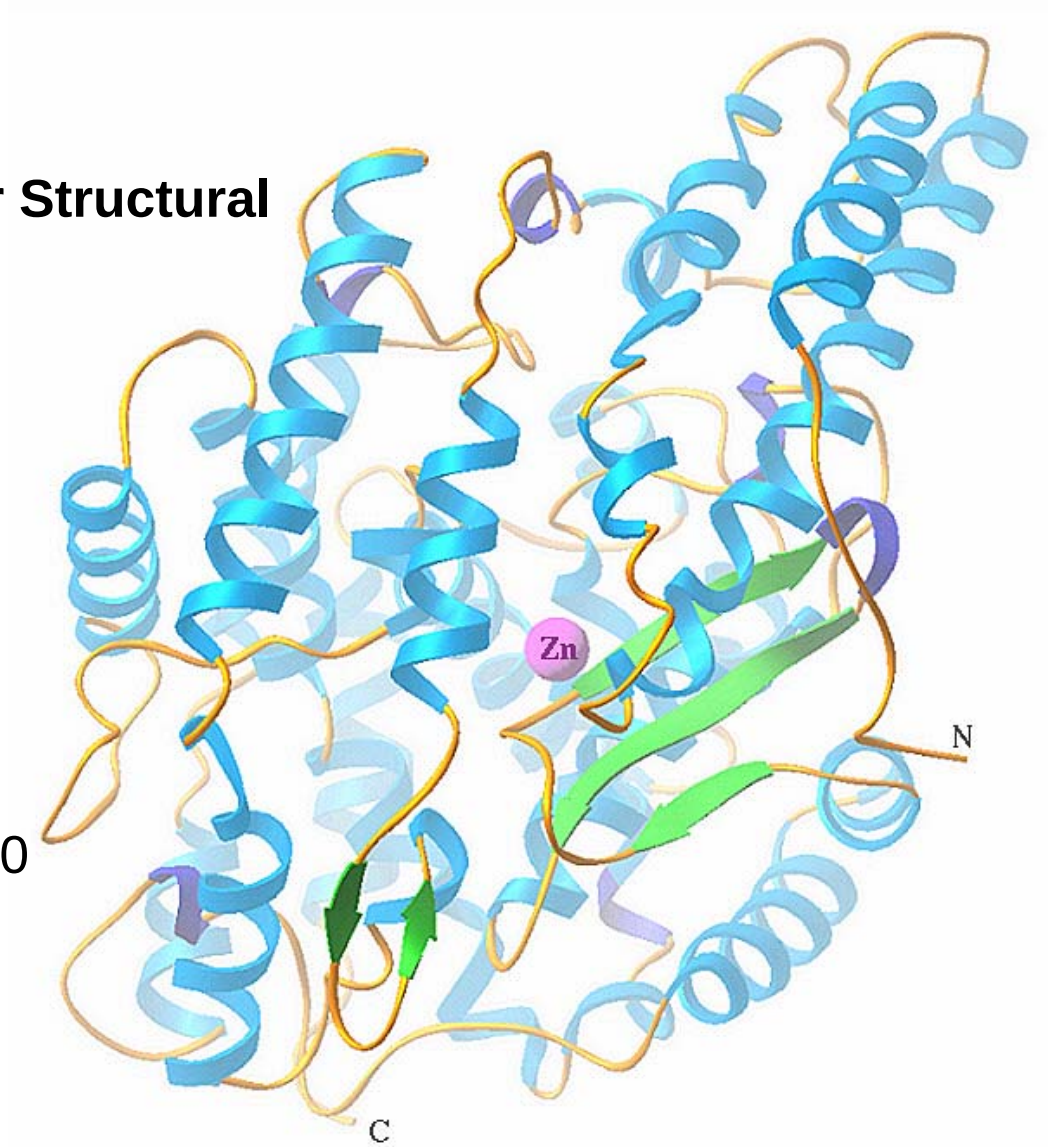
100 μm

Single crystals using microfluidic seeding



Data Collection - Structure Determination

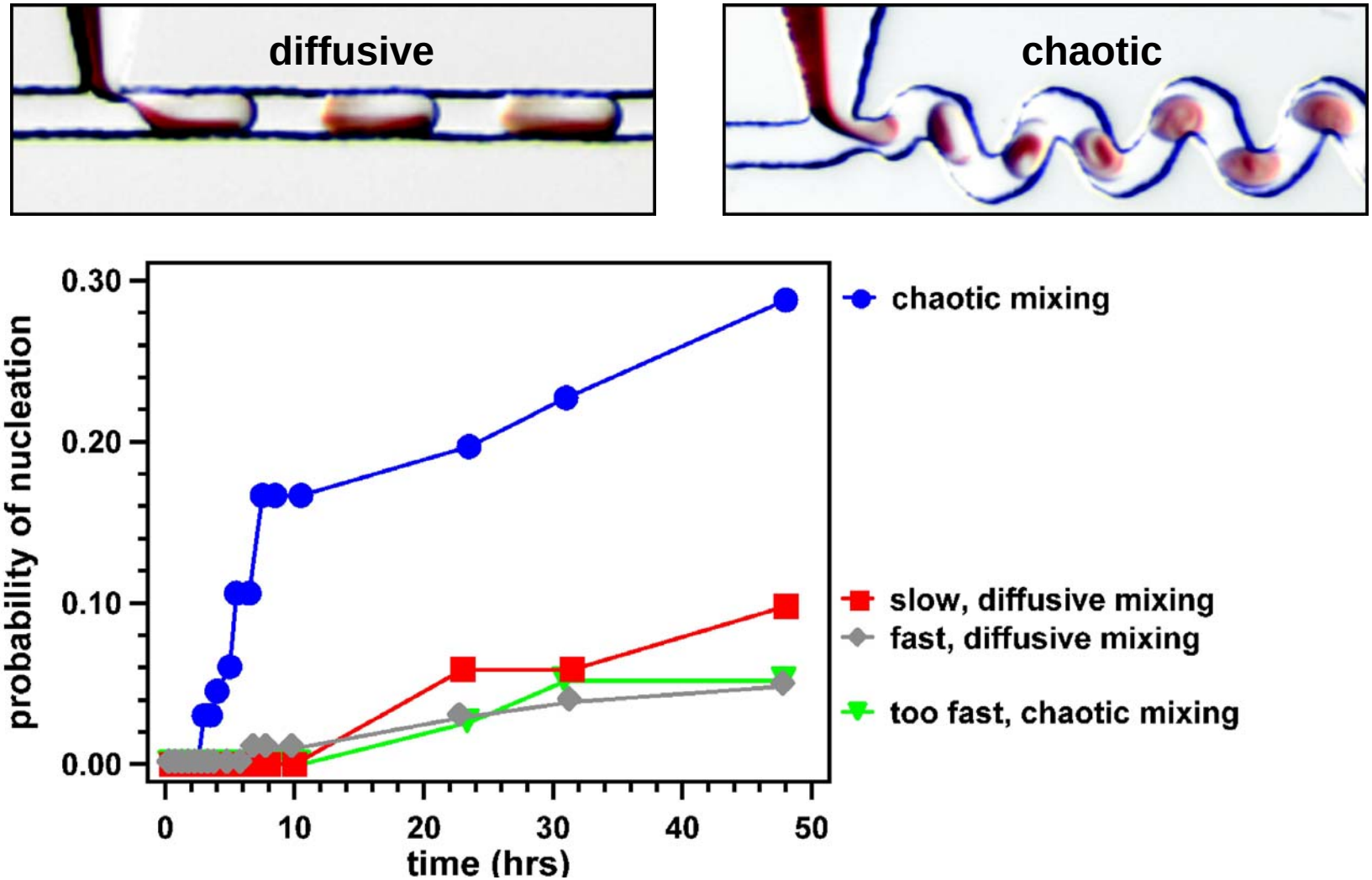
- metallopeptidase family M3
- selected by Midwest Center for Structural Genomics but unsolved and set aside



- solved by SAD technique
- 3.1 Å resolution
- Space group: P3121; Unit Cell
- Parameters a=b 119.50 c=248.90
- R-factor = 0.196, Rfree = 0.248
- Solvent Content: ~70%;

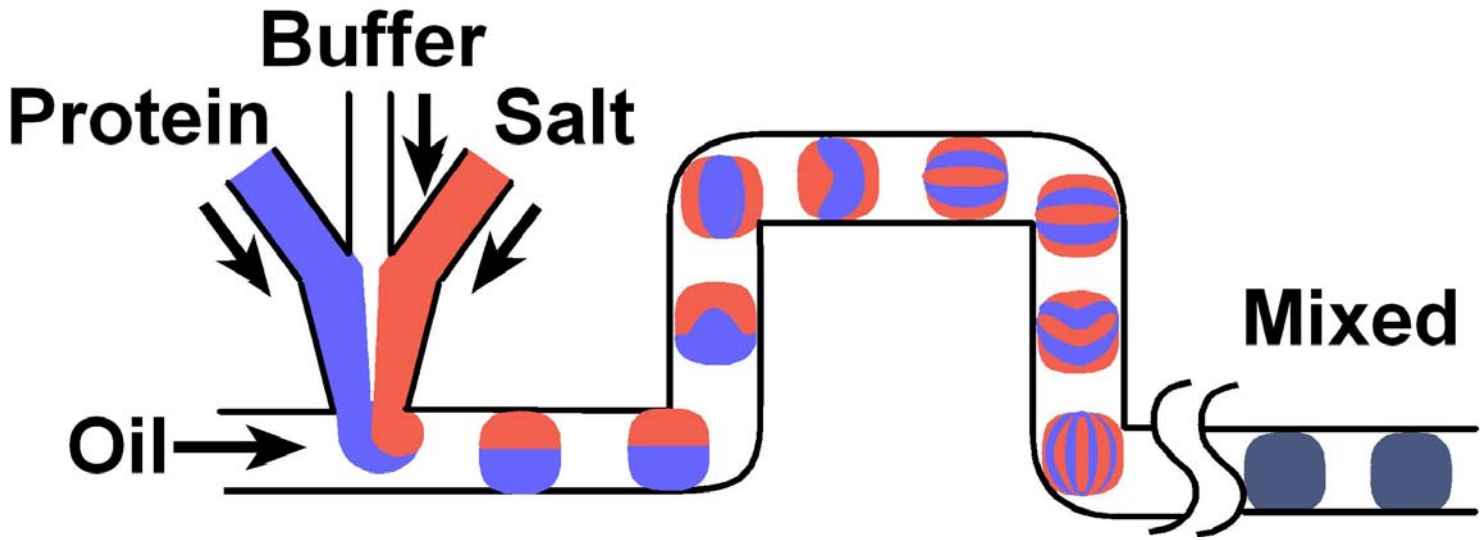
C. J. Gerdts and R.F.Ismagilov *Angew. Chem. Int. Ed.* 2006, 45,8156

Mixing is also Important for Protein Crystallization: Effective Nucleation by Slow Chaotic Mixing



Chen D. L. , Gerdts C. J., Ismagilov R. F. *J. Am. Chem. Soc. 2005*, 127, *9672-9673*.

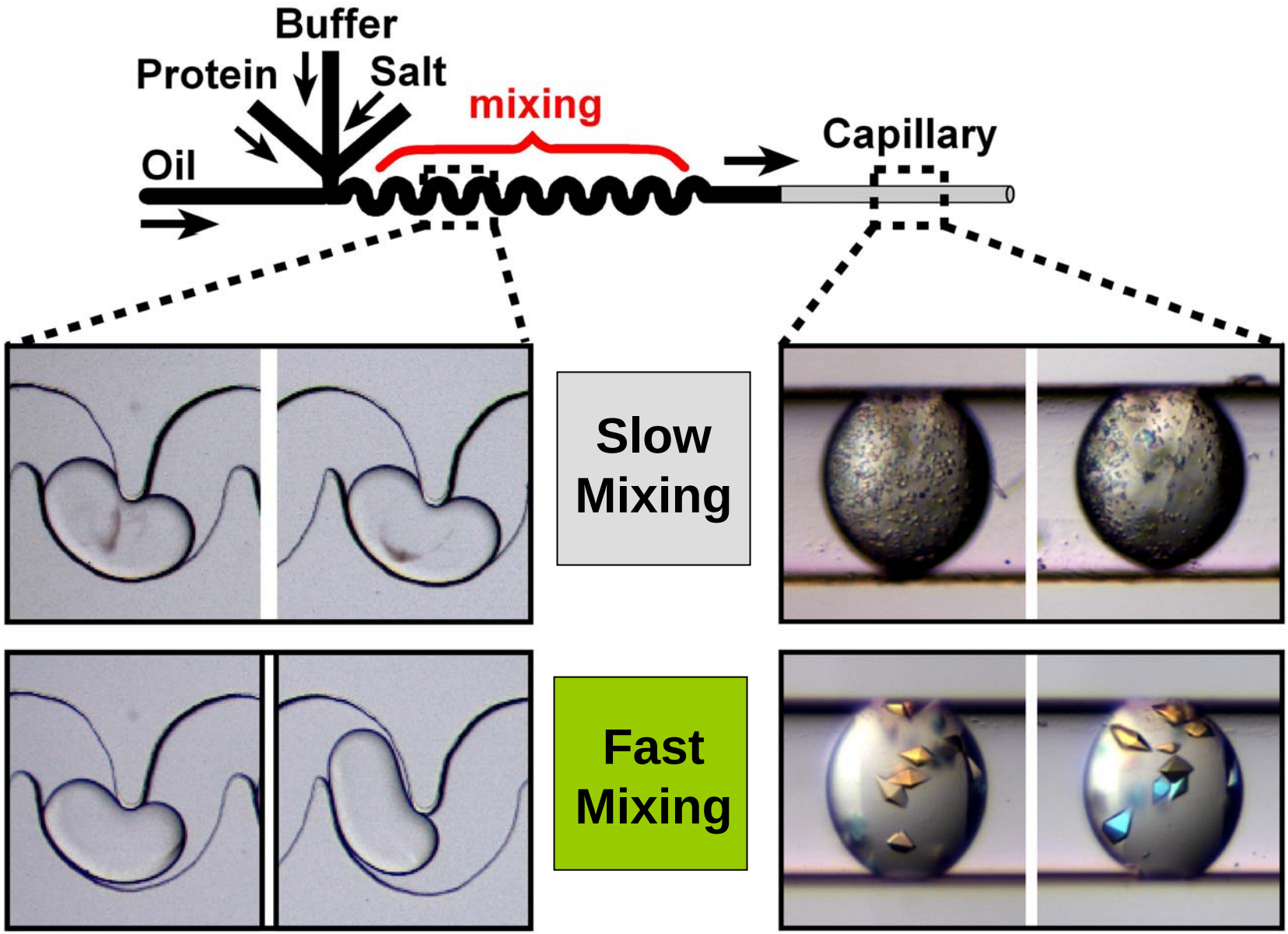
Understanding the Mixing Effect with a Chaotic Mixing Model



Assumption:
Only nucleation at interfaces is important

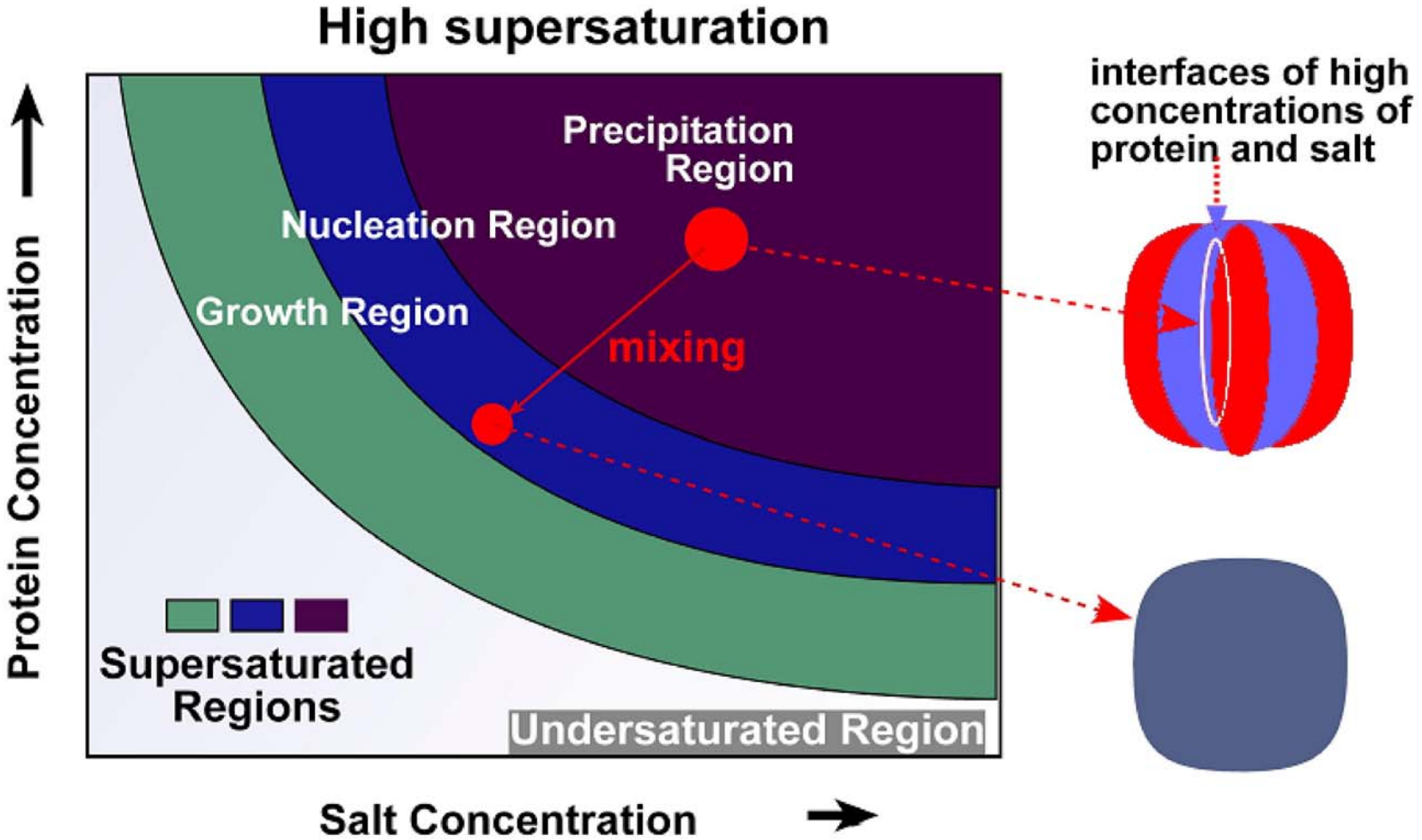
Slow Mixing	**Lifetimes of Interfaces are long**
Fast Mixing	**Lifetimes of Interfaces are short**

Experimental Results: Mixing Effect at High Supersaturation



Chen D. L. , Gerdts C. J., Ismagilov R. F. *J. Am. Chem. Soc. 2005*, 127, *9672-9673*.

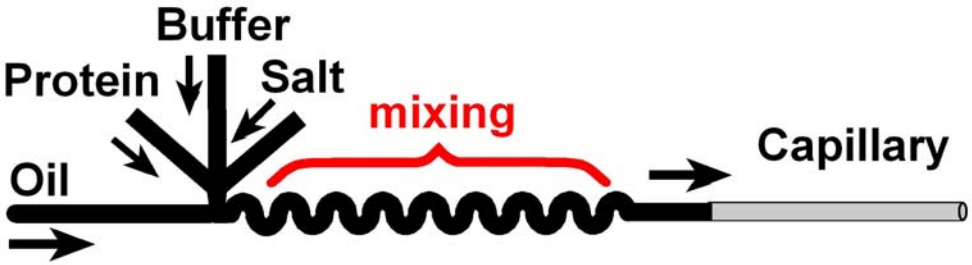
Protein Crystallization Phase Diagram: Time Dependent



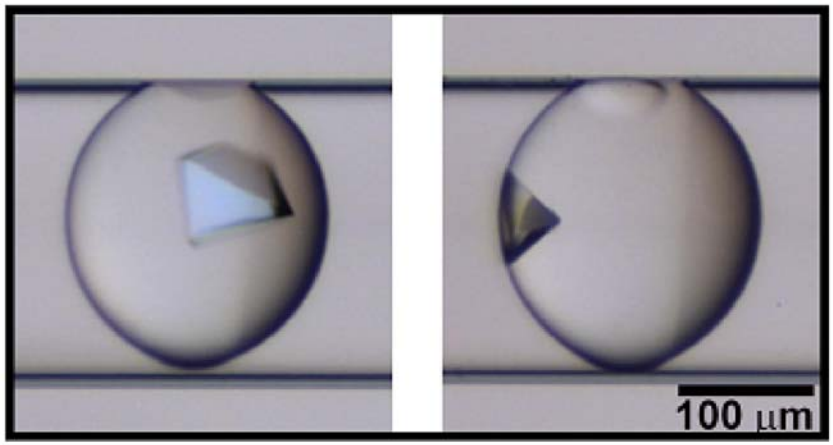
Slow Mixing: Precipitation happens faster than mixing

Fast Mixing: Mixing happens faster than precipitation

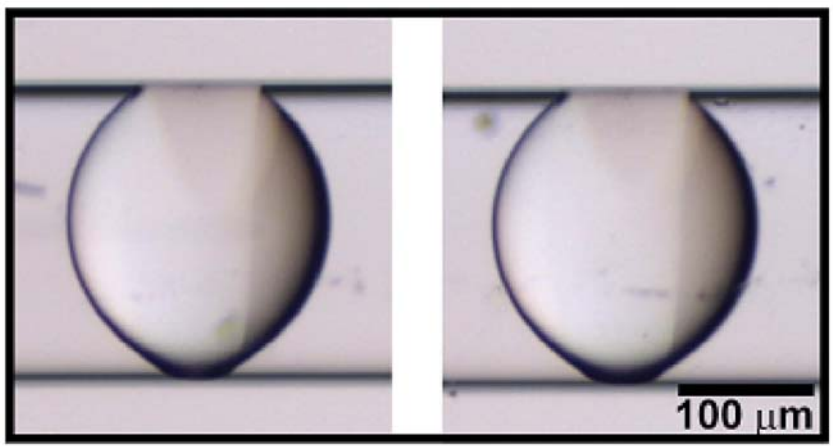
Experimental Results: Mixing Effect at Low Supersaturation



No crystals may mean improper mixing instead of a bad precipitant



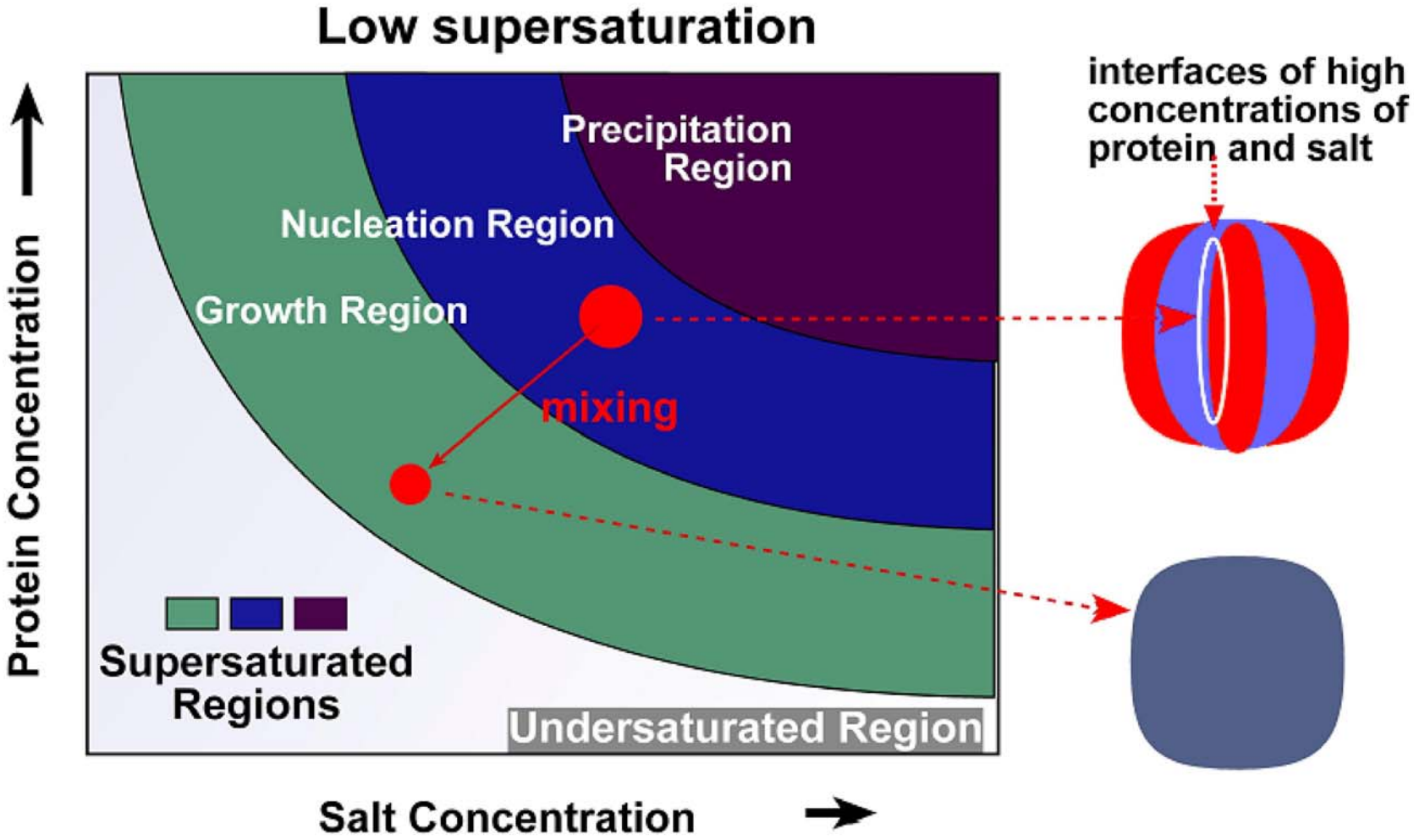
Slow Mixing



Fast Mixing

Chen D. L. , Gerdts C. J., Ismagilov R. F. *J. Am. Chem. Soc. 2005*, 127, *9672-9673*.

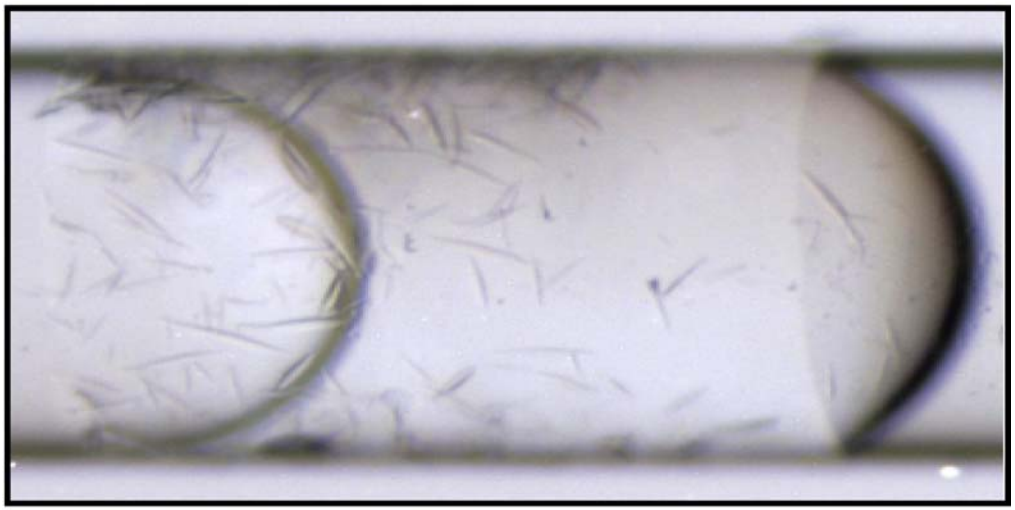
Protein Crystallization Phase Diagram: Time Dependent



Slow Mixing: Nucleation happens faster than mixing

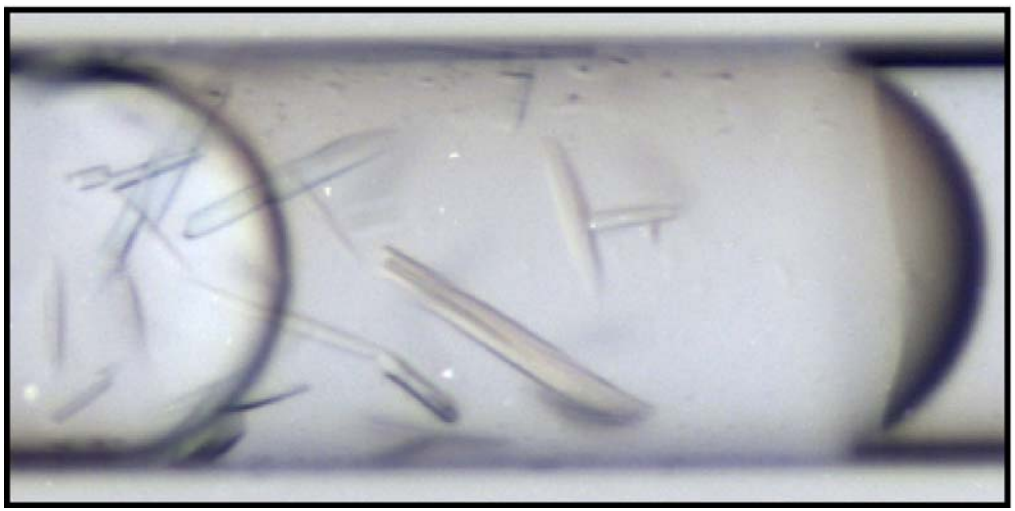
Fast Mixing: Mixing happens faster than nucleation

Mixing Effect in the Crystallization of a Novel Protein



Slow Mixing

many crystals



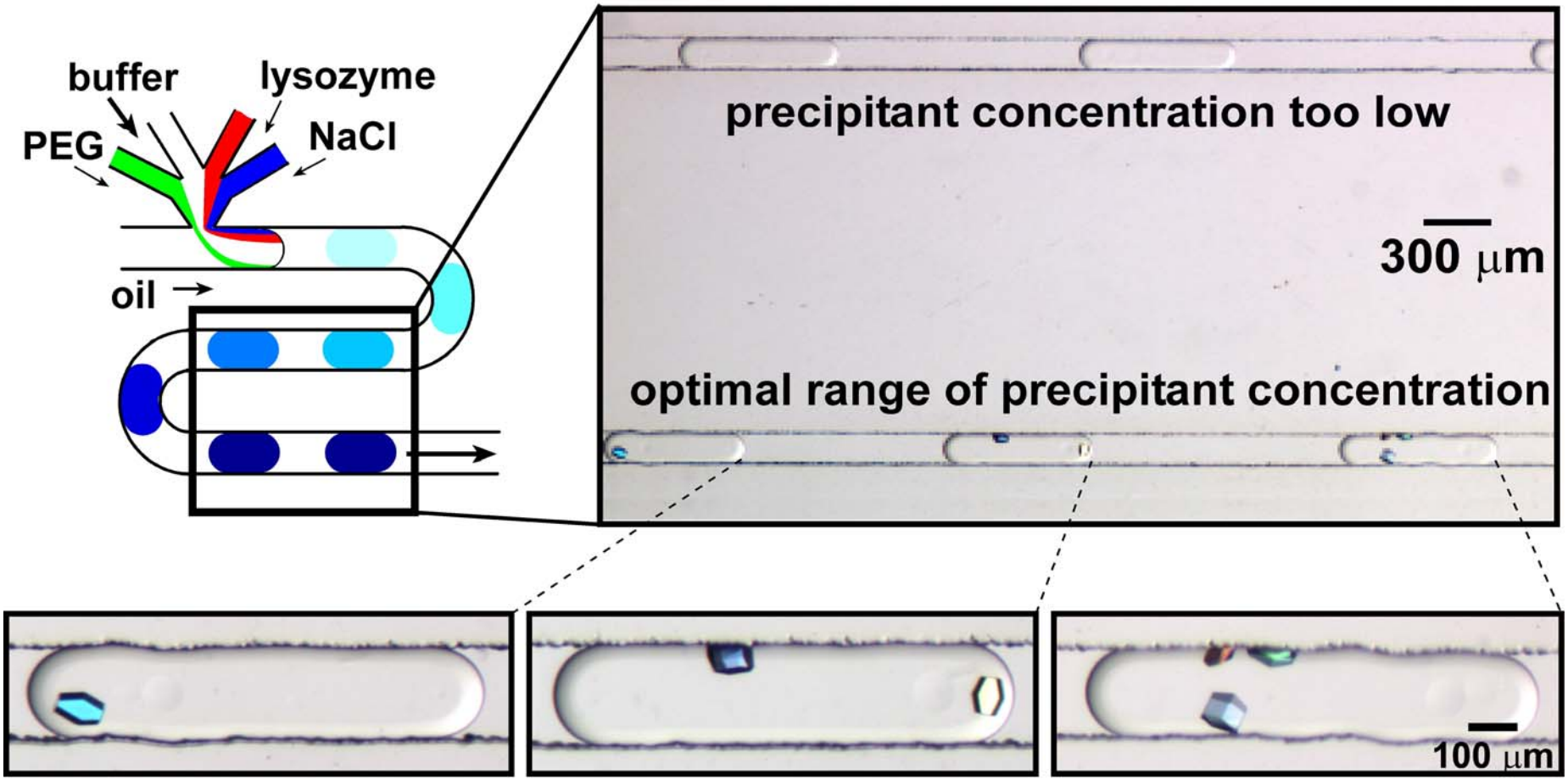
Fast Mixing

few crystals

ubiquination protein provided by Dr. Brenda Shulman, St. Jude Children's Research Hospital

Developing Microfluidic Tools to Screen Protein Crystallization Conditions

1. Gradient Screen of Crystallization Conditions

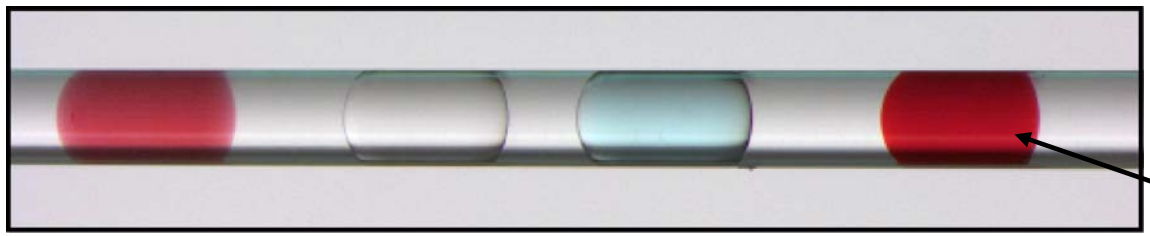


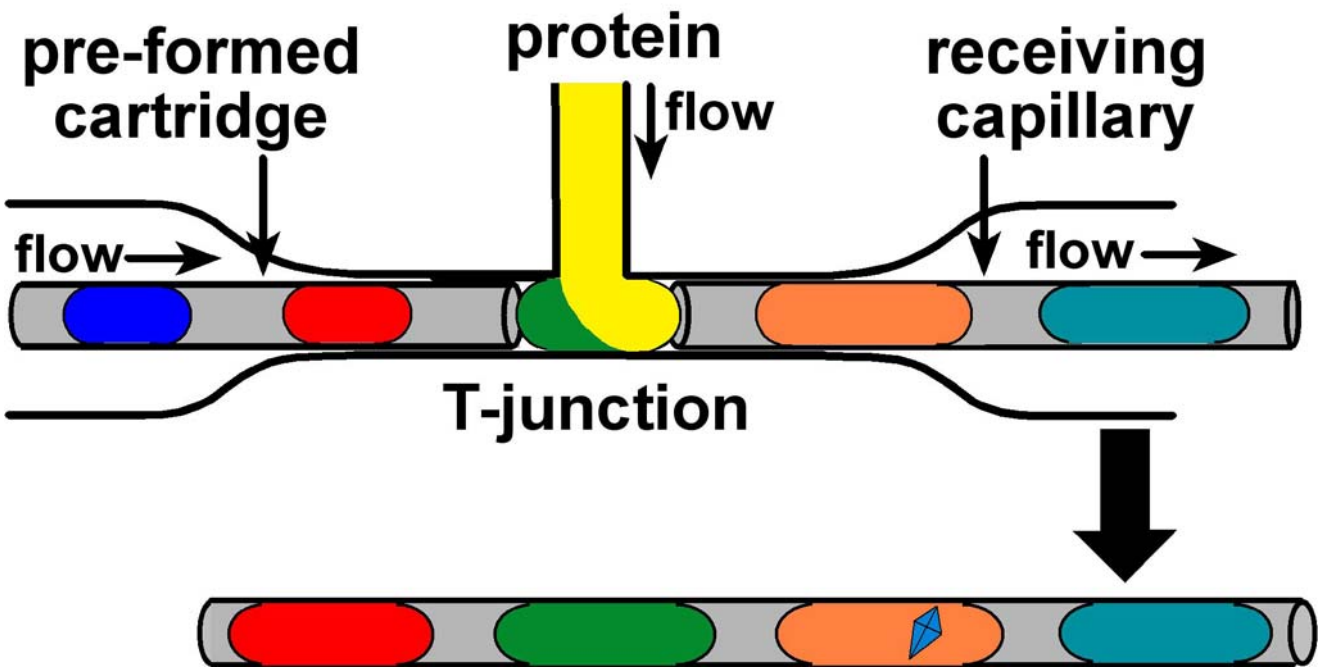
B. Zheng, L. S. Roach, R. F. Ismagilov *J. Am. Chem. Soc.* 2003, 125, 11174.

Developing Microfluidic Tools to Screen Protein Crystallization Conditions

2. Sparse Matrix Screen in Nanoliter Plugs

Pre-formed Cartridge





B. Zheng, R. F. Ismagilov *Angew. Chem.* 2005, 117, 2576.

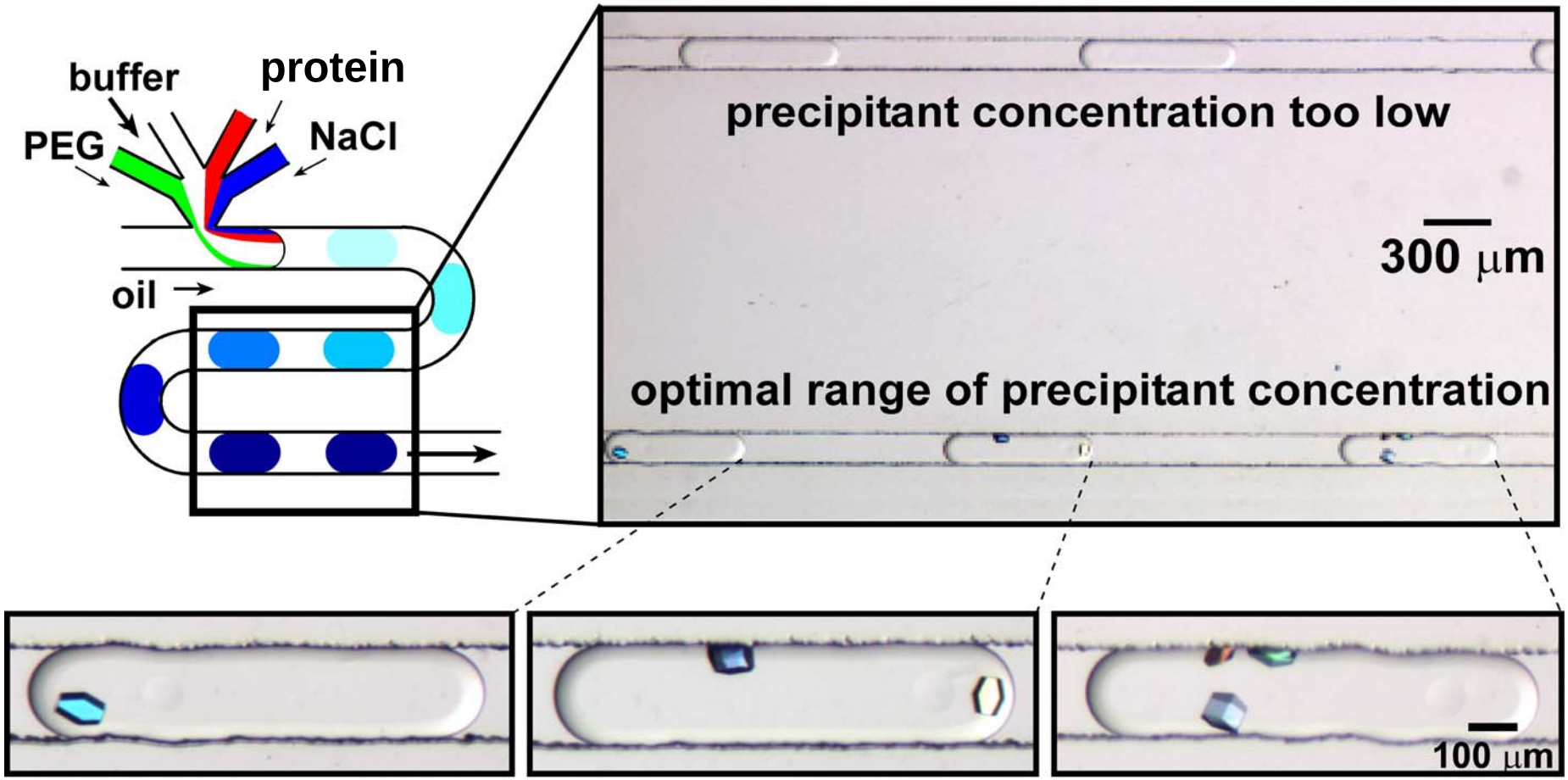
Crystallization of Membrane Proteins

- **Determining crystal structure of membrane protein: focus of major research efforts.**
  - **Important signaling functions**
  - **Targets of >50% drugs**

- **Crystallization of membrane protein: big challenge**
  - **Low in quantity, unstable over time.**
  - **Search of crystallization conditions: Broad and dense**

- **Two Challenges on handling membrane proteins**
  - **High viscosity**
  - **Low surface tension**

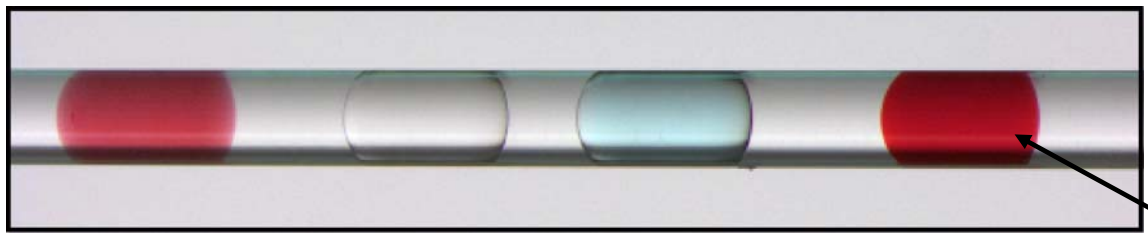
Gradient Screen of Crystallization Conditions



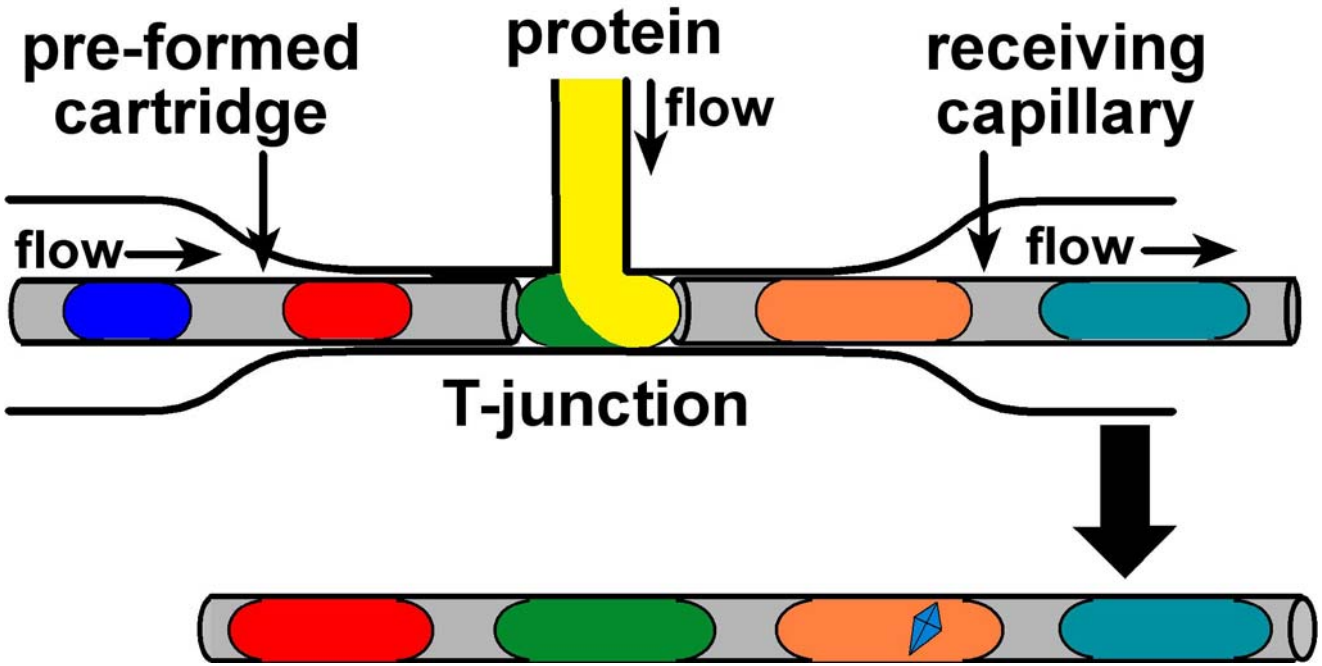
Dense but NOT Broad !

Sparse Matrix Screening in Nanoliter Plugs

Pre-formed Cartridge

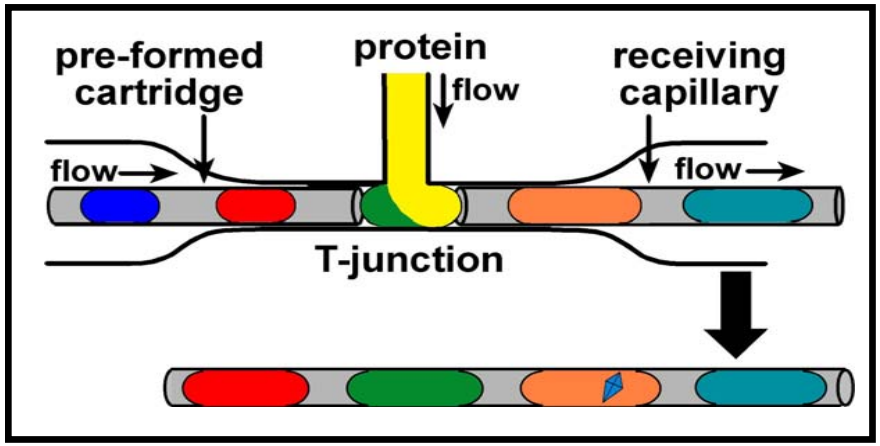


~ 10 nL



Broad but NOT Dense !

Hybrid Method: Combining Sparse Matrix and Gradient Screening



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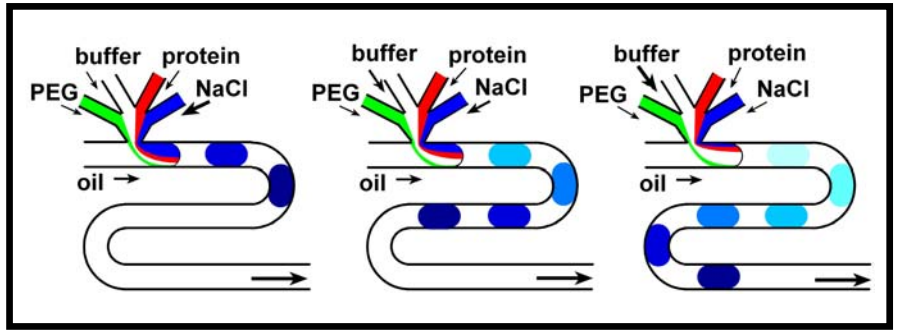
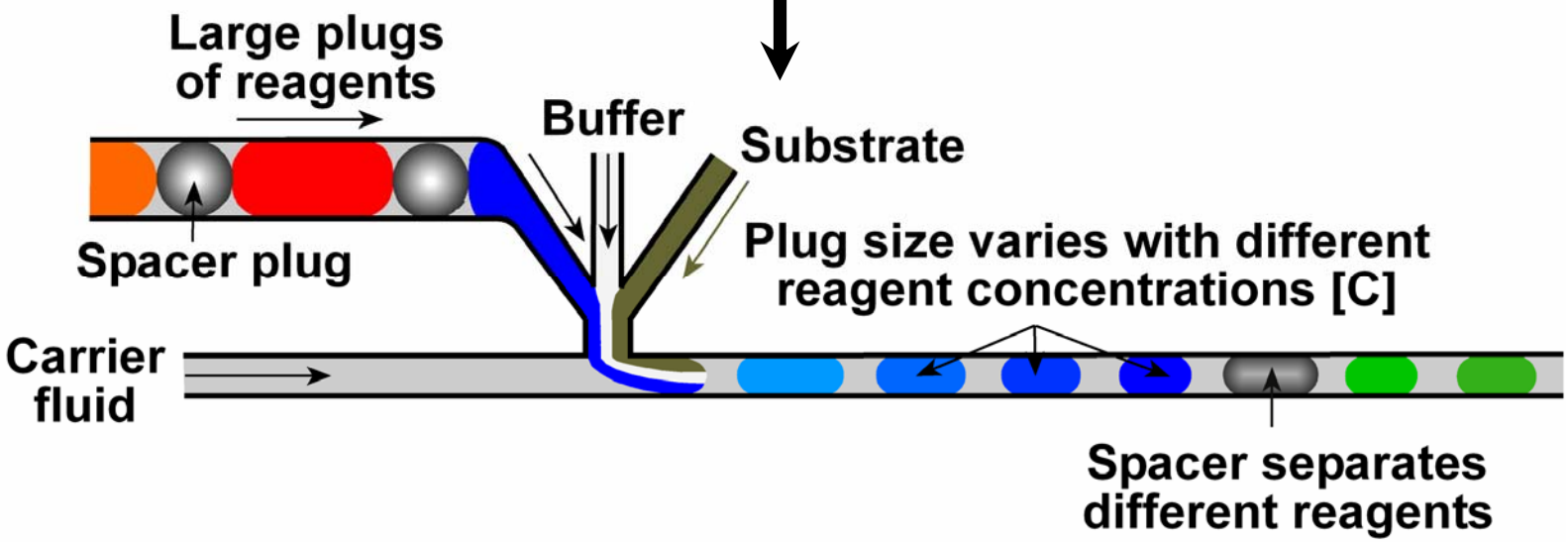
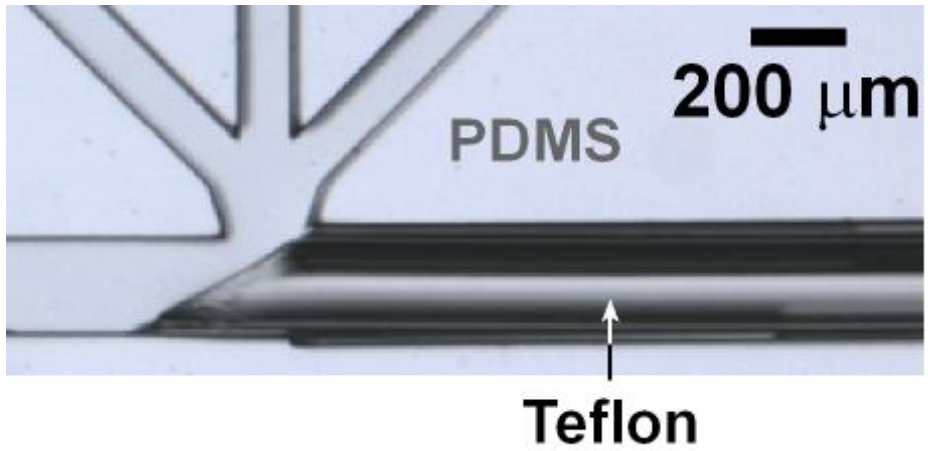




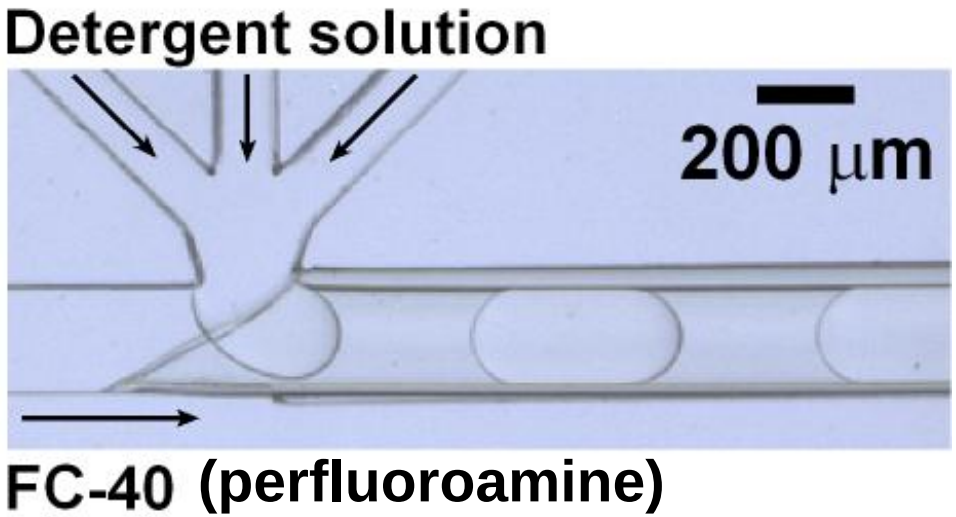
L. Li, D. Mustafi, Q. Fu, V. Tereshko, D.L. Chen, J.D. Tice, R.F. Ismagilov
PNAS, 2006, 103, 19243.

Crystallization of Membrane Proteins using Hybrid Method: Two Technical Developments Alleviate the Challenges

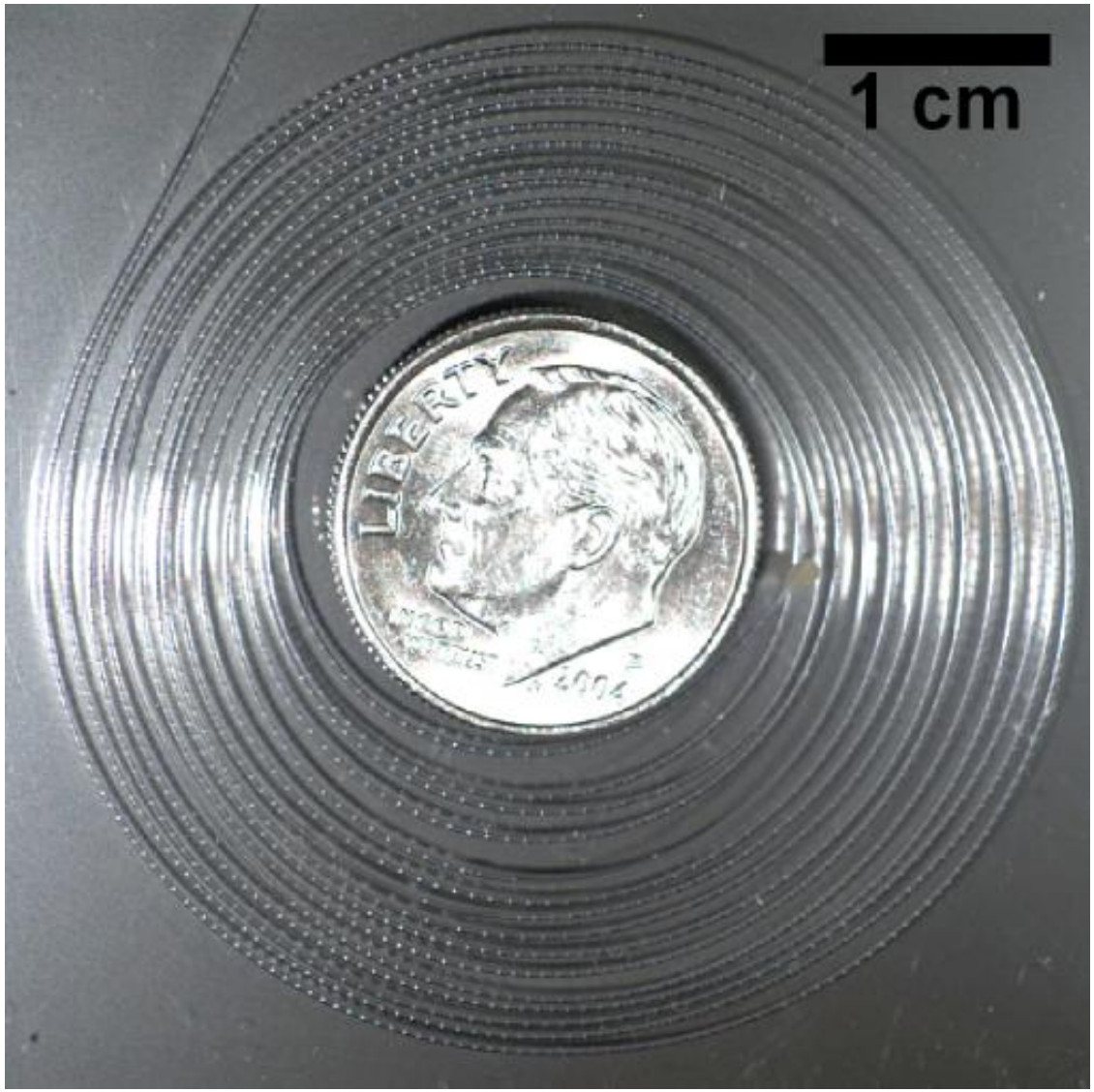
1. The use of **Teflon capillaries** for the formation, transport, and storage of plugs.



2. The use of **perfluoroamines** as carrier fluids

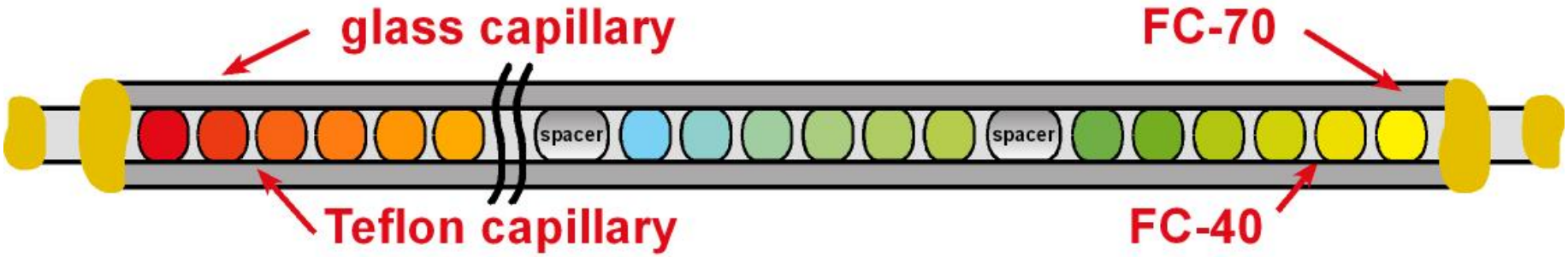


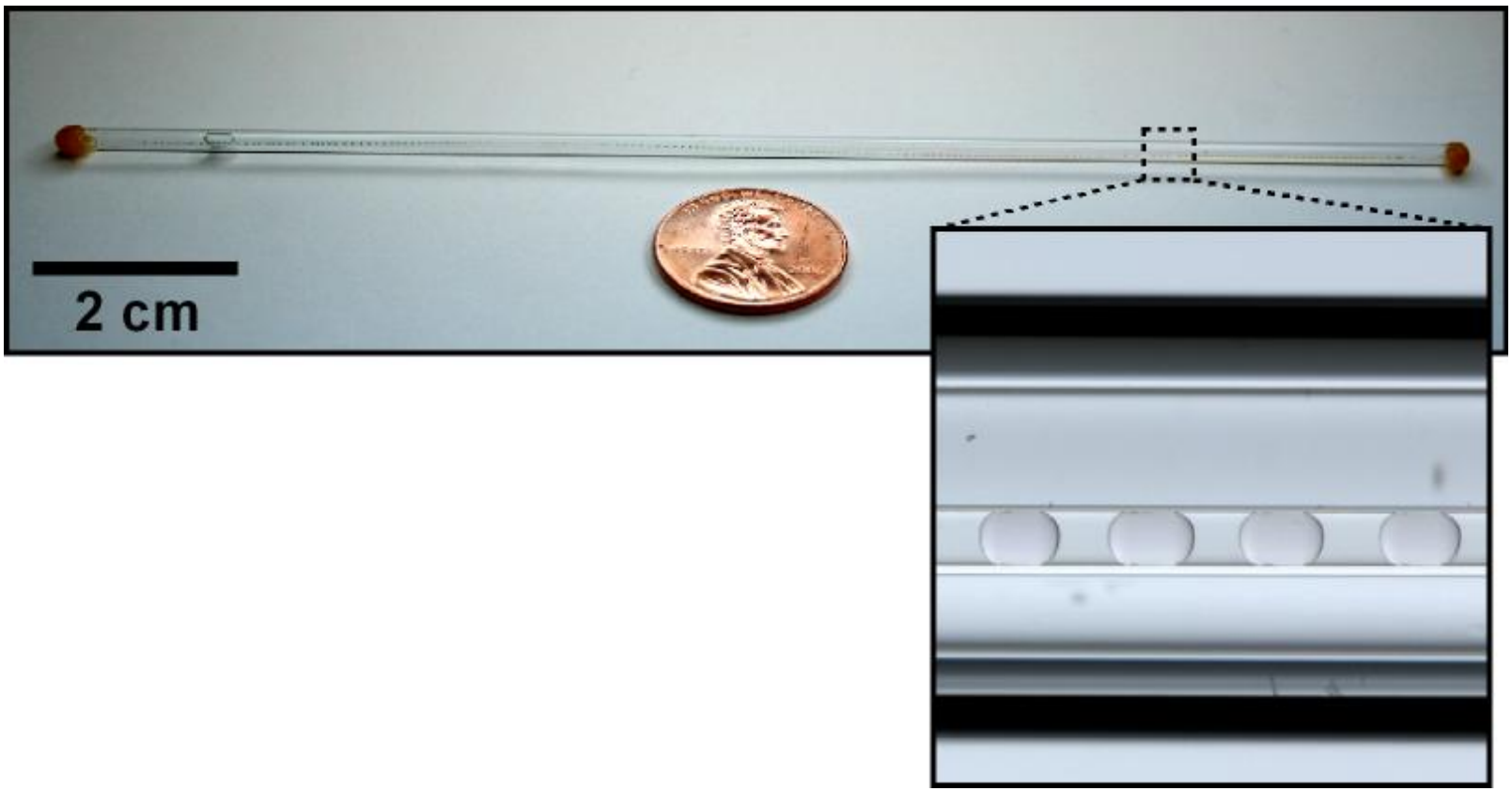
Membrane Protein Crystallization in Teflon Tubing: Compact Plug Storage



1 meter of tubing, ~1000 trials

Crystallization of Membrane Proteins using Hybrid Method: Long Term Plug Storage

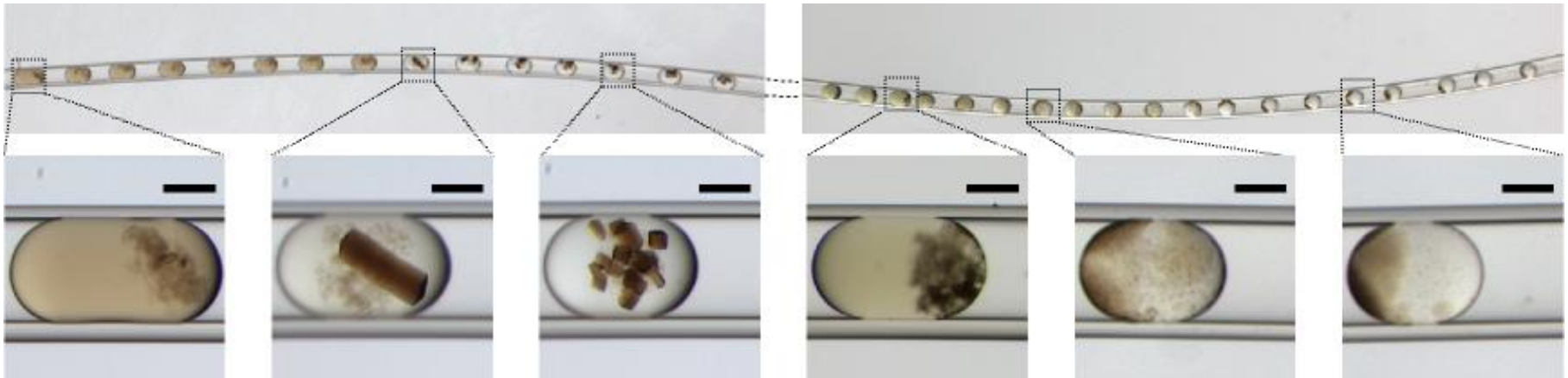




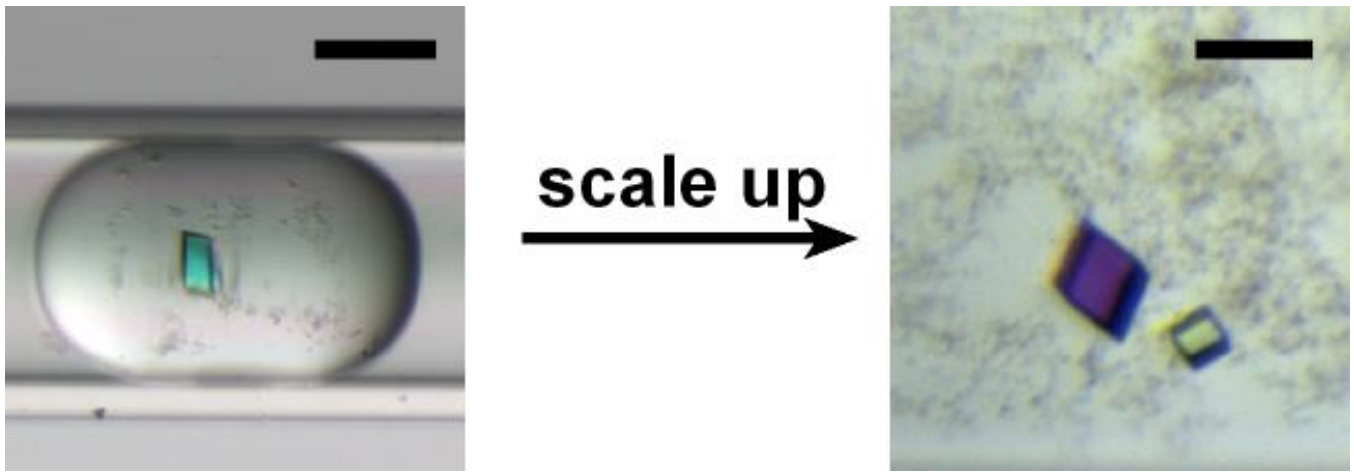
Crystallization of Model Membrane Proteins using Hybrid Method

Reaction Center

Precipitant #1 **Precipitant #2**

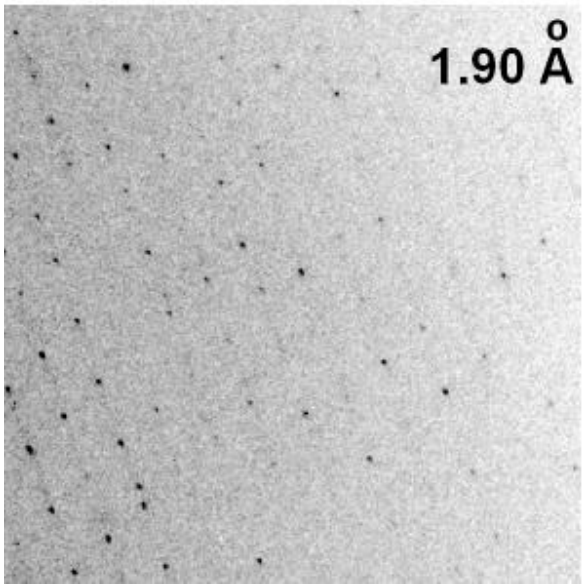


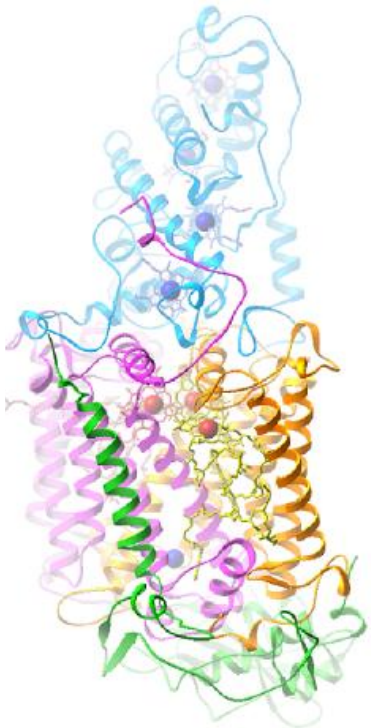
Porin



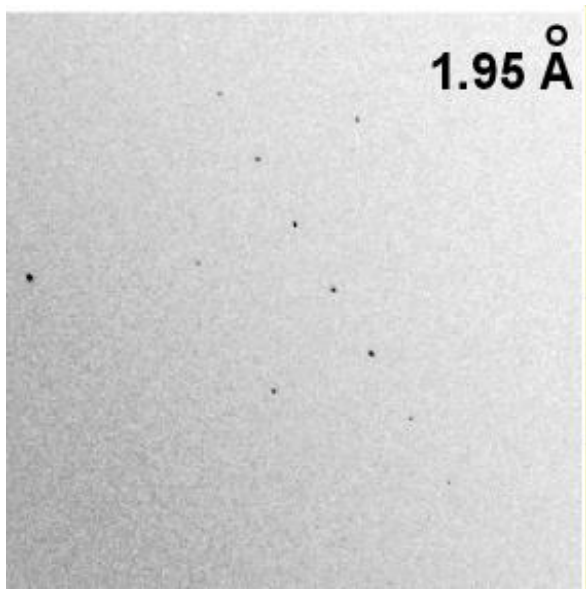
Diffraction of Membrane Protein Crystals Grown in the Hybrid Method

Reaction Center (*B. Viridis*)





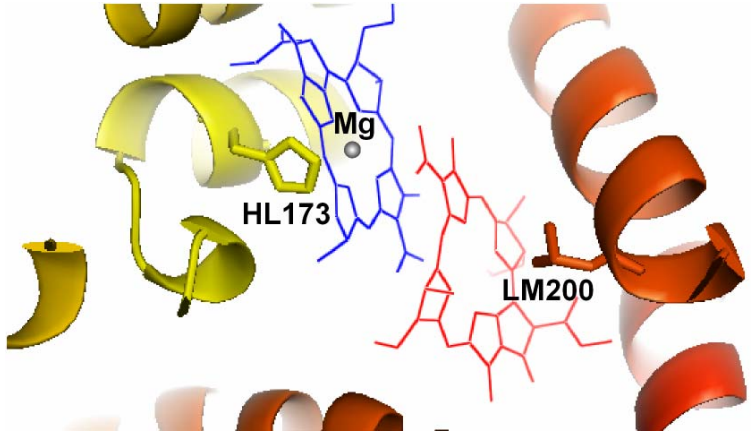
Porin (*R. Capsulatus*) in situ diffracion image



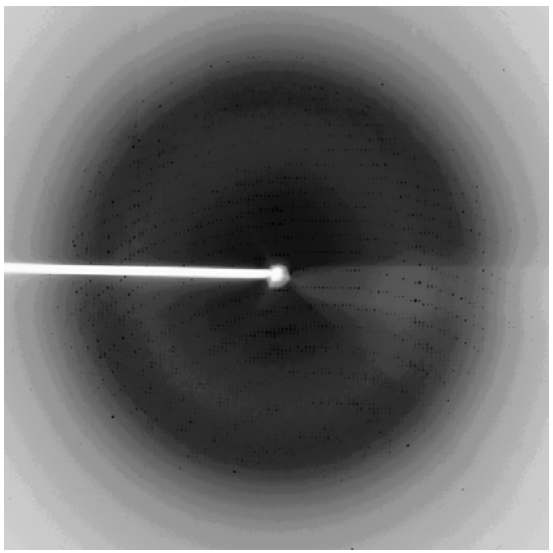
L. Li, D. Mustafi, Q. Fu, V. Tereshko, D.L. Chen, J.D. Tice, R.F. Ismagilov *PNAS,* 2006, 103, 19243.

Solving New Structures: A Continuous Endeavor

RC Mutant (*B. Viridis*)
Solved at 2.5 Å

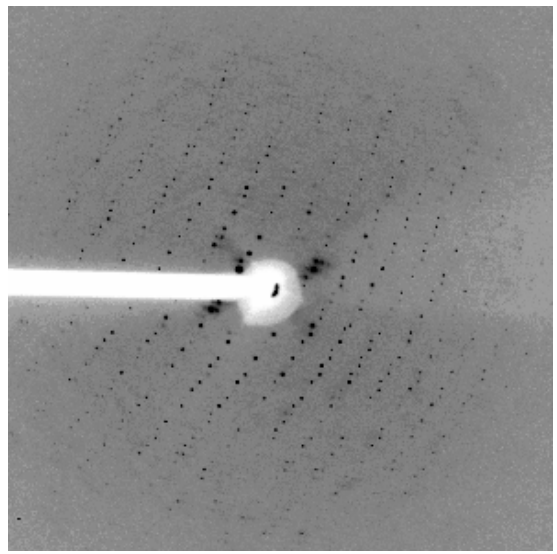


Target 1:



P4
2.9 Å
$\alpha=\beta=\gamma=90^{o}$,
a=183.9 Å,
b=183.9 Å,
c=113.9 Å.

Target 2:



P2
6.5 Å
$\alpha=\gamma=90^{o}$,
$\beta=101.5^{o}$,
a=171.3 Å,
b=232.7 Å,
c=331.7 Å.

Unpublished

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Protein Crystallization and Droplets:

Liang Li, Cory Gerdts, Delai Chen, Qiang Fu, Debarshi Mustafi

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Other Stories: James Boedicker, Meghan Bush

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